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Deoxynivalenol increases pro-inflammatory cytokine secretion and reduces primary bile acid transport in an inflamed intestinal *in vitro* co-culture model

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ABSTRACT

The fungal secondary metabolite deoxynivalenol (DON) that can contaminate cereal-based food products not only induces inflammation but also reduces bile acid absorption by a healthy human intestine. Bile acid malabsorption is commonly observed in individuals with an inflamed intestine. Here we studied the effects of DON on inflammation and primary bile acid transport using an *in vitro* model for an inflamed intestine. An inflamed intestinal *in vitro* model was established by co-culturing a Caco-2 cell-layer and LPS-pre-stimulated THP-1 macrophages in Transwells. We observed a decreased transport of 5 primary bile acids across inflamed co-cultures compared to healthy co-cultures but not of chenodeoxycholic acid. DON exposure further reduced the transport of the affected primary bile acids across the inflamed co-cultures. DON exposure also enhanced the secretion of pro-inflammatory cytokines in the inflamed co-cultures, while it did not increase the pro-inflammatory cytokines secretion from LPS-pre-stimulated THP-1 monocultures. Exposure of Caco-2 cell-layers to pro-inflammatory cytokines or THP-1 conditioned media partly mimicked the DON-induced effects of the co-culture model. Local activation of intestinal immune cells reinforces the direct pro-inflammatory effects of DON on intestinal epithelial cells. This affects the bile acid intestinal kinetics in an inflamed intestine.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic intestinal disorders in which a dysregulated intestinal immune response is a key factor (Attauabi, Zhao, Bendtsen, & Burisch, 2021; Pithadia & Jain, 2011). The aetiology of IBD is multifactorial and is manifested in complex symptoms, such as changes in intestinal microbial composition, intestinal tissue damage, and abdominal pain (Maloy & Powrie, 2011; Sands, 2004). A common, but often ignored symptom of IBD is primary bile acid malabsorption by the small intestine (Fitzpatrick & Jenabzadeh, 2020). The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized and conjugated with glycine or taurine in the liver which gives rise to either glyco-CA (GCA), tauro-CA (TCA), glyco-CDCA (GCDCA) and tauro-CDCA (TCDCA) bile acids (Staels & Fonseca, 2009). After secretion via the bile into the small intestinal lumen, the majority of bile acids, are subsequently reabsorbed in the ileum by bile acid active transporters (i.e., the apical sodium-dependent bile acid transporter ASBT and the organic solute transporter OST) and circulate back to the liver via the portal vein, where they are recycled for another entero-hepatic cycle (Hofmann, 1977). Decreased bile acid reabsorption in the ileum consequently leads to an increased loss of bile acids via feces and a reduction of the systemic bile acid pool, both of which are common symptoms of IBD (Roda et al., 2019).

The trichothecene toxin deoxynivalenol (DON) is a fungal secondary metabolite that can contaminate cereal-based food. The provisional maximum tolerable daily intake (PMTDI) established by the European Food Safety Authority (EFSA) is 1 μg/kg bw per day, while the average dietary DON exposure in humans is up to 0.2–14.5 μg/kg bw per day estimated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (EFSA, 2017; Joint, Additives, & Organization, 2011). After consumption, DON is rapidly absorbed through the intestinal small intestinal mucosa, where DON encounters not only the cells present in the

Abbreviations: DON, Deoxynivalenol; IBD, Inflammatory Bowel Diseases; CA, Cholic Acid; CDCA, Chenodeoxycholic acid; GCA, glyco-CA; TCA, tauro-CA; GCDCA, glyco-CDCA; TCDCA, tauro-CDCA; EFSA, European Food Safety Authority; ELISA, Enzyme-Linked Immuno Sorbent Assay; MEM, Minimum Essential Medium Eagle; TEER, Transepithelial electrical resistance; PMA, Phorbol 12-myristate 13-acetate.

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intestinal epithelium but also the immune cells that are abundantly present in the underlying intestinal lamina propria (Ponce de León-Rodríguez, Guyot, & Laurent-Babot, 2019; Sergent et al., 2006). During this process, DON stimulates pro-inflammatory cytokine production in the intestinal mucosa (EFSA, 2017). From *in vitro* studies it is known that DON stimulates the secretion of pro-inflammatory cytokines IL-6 and IL-8 by human intestinal epithelial Caco-2 and HIEC-6 cell lines (Pomothy et al., 2021; Van De Walle, Romier, Larondelle, & Schneider, 2008). In addition, DON also increases the pro-inflammatory cytokine IL-1β and IL-8 secretion by human monocyte U937 cells and peripheral blood mononuclear cells (PBMCs) (Gray & Pestka, 2007; Islam, Gray, & Pestka, 2006). Animal studies showed that DON induced intestinal inflammation at a dose of 10 μg/kg bw per day, which is within the range of estimated human dietary DON exposure levels (Joint, Additives, & Organization, 2011; Vignal et al., 2018). Thus, DON is known as an pro-inflammatory agent in a healthy intestine.

The role of DON in inflamed intestinal mucosa might be different. Emerging evidence shows that DON inhibits the activation of the TLR4/NF-κB signalling pathway induced by porcine epidemic diarrhoea virus in porcine alveolar macrophages (Liu, Wang, He, Ge, & Huang, 2022). TLR4 is an essential receptor that responds to bacterial lipopolysaccharide (LPS), which activates the pro-inflammatory NF-κB signalling pathway and causes intestinal inflammation in humans (Candelli et al., 2021). The inhibition of the activation of the TLR4/NF-κB signalling pathway by DON may affect the pro-inflammatory cytokine production in an inflamed intestine. Furthermore, the inflammatory state of the intestinal mucosa may affect the primary bile acid transport in inflamed intestine following DON exposure. We have previously shown that DON exposure decreases the transport of a mixture of ten conjugated bile acids across cell layers of differentiated Caco-2 monocultures by decreasing the ASBT gene expression (Wang et al., 2022). In both Caco-2 and IEC-6 cells ASBT gene expression is downregulated following exposure to IL-1β and TNFα, but not in response to IL-6 exposure (Chen et al., 2002). These three pro-inflammatory cytokines are secreted by immune cells residing in the intestinal mucosa in inflammatory state. While many cytokines are secreted by intestinal immune cells, Caco-2 cells mainly respond to IL-1β and TNFα and show downregulation of ASBT gene expression (Chen et al., 2002). Thus, IL-1β and TNFα are selected as pro-inflammatory cytokines for the current study.

In vitro intestinal models are essential tools to study the (patho) physiology of intestinal disorders and to better understand the interaction of chemicals with and between intestinal cells. Caco-2 cell layers are the most widely used intestinal model for transport and intestinal toxicity studies (Calatayud, Gimeno-Alcañiz, Devesa, & Vélez, 2015; Kämpfer et al., 2017; Kaulmann, Legay, Schneider, Hoffmann, & Bohn, 2016; Ponce de León-Rodríguez et al., 2019; Zhang et al., 2017). *In vitro* co-cultures of Caco-2 cell layers with primary cells like human peripheral blood mononuclear cells (PBMC) and human monocyte cell line THP-1 enable the development of immune-active models mimicking *in vivo* human intestinal mucosa both in healthy and inflamed states (Calatayud et al., 2015; Kämpfer et al., 2017; Kaulmann et al., 2016; Zhang et al., 2017). However, these inflamed intestinal co-cultures are characterized by leaky barriers, which are not suitable for transport studies. To this end, we aimed to develop an inflamed co-culture model that maintains barrier integrity comparable to that of the healthy co-cultures. Upon phorbol 12-myristate 13-acetate (PMA) treatment, THP-1 monocytes differentiate into macrophages, which are the most numerous immune cells in the intestinal mucosa (Lund, To, O'Brien, & Donnelly, 2016; Ponce de León-Rodríguez et al., 2019). The use of THP-1 macrophages improves the reproducibility of the experiments over PBMC, due to their homogeneous genetic background. Thus, in this study, we explored the effect of DON on pro-inflammatory cytokine secretion and primary bile acid transport in an inflamed intestinal *in vitro* model, which was established using intestinal epithelial Caco-2 cell-layers and LPS-pre-stimulated THP-1 macrophages grown in Transwells. Pro-inflammatory cytokines were detected by Enzyme-Linked Immune-Sorbent Assay (ELISA). Primary bile acid transport was quantified by using an LC-MS/MS method.

2. Materials and methods

2.1. Cell cultures

Human colon carcinoma Caco-2 cells (passage number 10–25) were grown at 37 °C with 5% CO_2 in growth medium composed of Minimum Essential Medium Eagle (MEM) (Gibco BRL, Breda, the Netherlands), supplemented with 20% heat-inactivated fetal calf serum (FCS), 1% pyruvate and 1% penicillin/streptomycin/glutamine (Gibco BRL). Cells were sub-cultured at 50–60% confluence by using 0.05% trypsin (Gibco BRL). Human immune THP-1 cells (passage number 18–35) were grown at 37 °C with 5% CO_2 in growth medium composed of RPMI1640 (Gibco BRL), supplemented with 10% FCS and 1% penicillin/streptomycin (Gibco BRL). Cells were exposed to chemicals using their respective growth media unless specified differently.

2.2. Cell viability

Cytotoxicity of DON on naïve (i.e. non LPS pre-stimulated) or LPS-pre-stimulated THP-1 macrophages was evaluated by the WST-1 assay. Briefly, THP-1 monocytes were exposed to 50 ng/ml PMA (Sigma-Aldrich, St. Louis, MO, USA) at 1.5×10^4 cells/well in 96-well plate for 24 h to differentiate them into macrophages. After removing PMA, the THP-1 macrophages were pre-exposed for 2 h for which 0.5% PBS or 10 ng/ml LPS was added to the exposure medium, then DON (0–12.5 μM) was added in the exposure medium and the cells were incubated for another 4 h. Subsequently the cells were incubated with WST-1 reagent 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) (Sigma-Aldrich) for 1 h. For this the WST-1 solution was added at 10% of the medium volume and the absorbance was measured at 440 nm and 620 nm with a SpectraMax M2 microplate reader (Molecular devices, San Jose, CA, USA) up to 60 min. 0.1% Triton X-100 (Sigma-Aldrich) was used as the positive control. Data were obtained by subtracting the 620 nm signal from the 440 nm signal. The cell viability was expressed as percentage of the solvent control group.

2.3. In vitro healthy and inflamed co-culture models

Caco-2 cells were seeded at 2×10^5 cells/insert on the apical side of 12-well polycarbonate or polyethylene terephthalate membrane inserts with a 0.4 μm pore size (Corning Costar, Schnelldorf, Germany) and maintained in culture for 18 days. The morphology of differentiated Caco-2 cells in Transwells using the comparable cultural protocol is shown in previous publications (Grouls, van der Zande, de Haan, & Bouwmeester, 2022; Kulthong et al., 2021). The cells were maintained in MEM-based Caco-2 culture medium and medium was refreshed every other day. The medium in the basolateral compartment was gradually changed from MEM-based Caco-2 medium to RPMI1640-based THP-1 culture medium during the 18 days of culturing (for details see Table S1). Transepithelial electrical resistance (TEER) was measured twice a week with a Millicell® ERS-2 Epithelial Volt-Ohm Meter (Millipore, Amsterdam, the Netherlands) during the 18 days. TEER values were expressed as Ω cm^2 and are shown in Fig. S1.

Healthy and inflamed co-culture models were developed using the 18-day differentiated Caco-2 cells and THP-1 macrophages. The healthy co-culture was established based on the protocol from Kampfer et al. (Kämpfer et al., 2017). Briefly, THP-1 monocytes were exposed to 50 ng/ml PMA at 3.0×10^6 cells/flask in T25 flask for 24 h, then detached using Accutase (Sigma-Aldrich) and re-seeded at the bottom of 12-well plates at 1.8×10^5 cells/well and allowed to settle for 1.5 h for re-attaching. The inserts with the 18 days cultured Caco-2 cells were then added in the 12-well plates to form the healthy co-culture (Fig. 1A).

To emulate inflamed conditions in the co-culture, 1.8×10^5 cells/

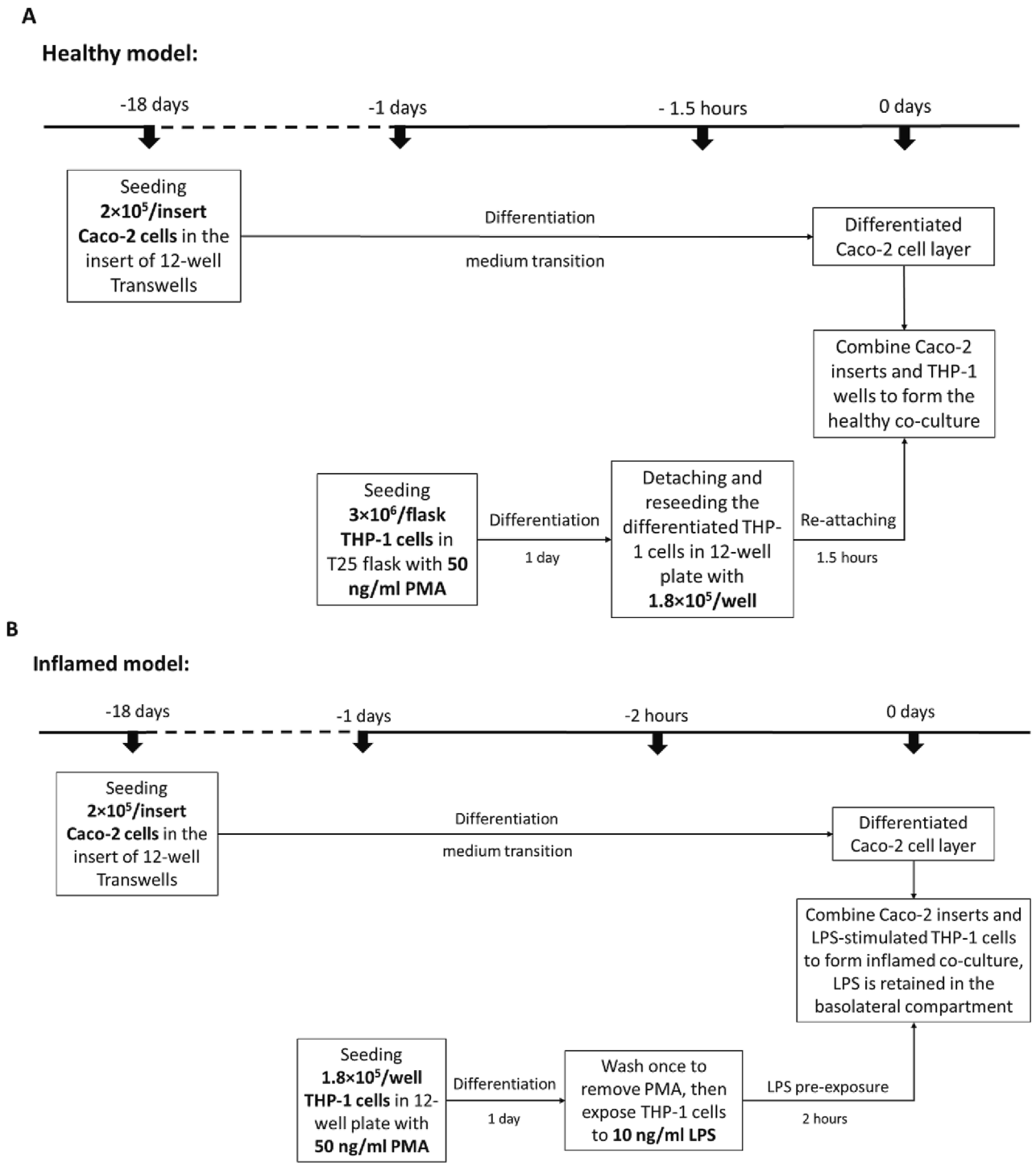

Fig. 1. Overview of creating the *in vitro* healthy (A) and inflamed (B) co-culture models.

well THP-1 monocytes were seeded in the 12-well plate with 50 ng/ml PMA for 24 h. After removing PMA, the differentiated THP-1 macrophages were pre-exposed to 10 ng/ml LPS for 2 h to induce an inflamed state (Fig. 1B). Subsequently, the inserts with 18 days cultured Caco-2 cells were directly added to the wells with the LPS-pre-stimulated THP-1 macrophages to form the inflamed co-culture. The LPS was retained in the basolateral compartment of the inflamed co-cultures during all subsequent exposure studies.

2.4. Caco-2 cell layer barrier integrity in healthy and inflamed co-culture models

The inserts with differentiated Caco-2 cell layers were added to the THP-1 macrophages (Fig. 1), following that the integrity of Caco-2 cell layers in the healthy and inflamed co-cultures was assessed by using TEER and Fluorescein transport measurements. Healthy or inflamed co-cultures were exposed to the control exposure conditions (i.e., 0.5% DMSO that was used as a solvent for DON) for 48 h. TEER measurements were performed at 4, 24 and 48 h following combining the Caco-2 and THP-1 macrophages. TEER values of the inflamed co-cultures were

expressed as percentage of healthy co-culture. Fluorescein transport was measured at 48 h only. For this the DMSO exposure medium was removed by rinsing the cell layers with Hank's balanced salt solution (Gibco BRL) supplemented with 10 mM HEPES (transport medium). After 30 mins of incubation in transport medium, 25 nmol Fluorescein (Sigma-Aldrich, 46955) in 0.5 mL transport medium was added to the apical cell compartment. After 1 h of incubation the amount of fluorescence was measured in the basolateral compartment with a SpectraMax M2 microplate reader. The excitation and emission wavelengths were 490 and 520 nm, respectively. The data are presented as the percentage of total fluorescein.

2.5. Mixed primary bile acid transport assay

Primary bile acid transport studies were performed in several study designs. Firstly, healthy or inflamed co-cultures were exposed to 0.5% DMSO or DON (0.5 or 2.5 μM) in both apical and basolateral compartments for 48 h. Secondly, primary bile acid transport was study using cell layers of Caco-2 monocultures were exposed to 2.5 ng/ml IL-1β + 5 ng/ml TNFα (low IL-1β + TNFα), 10 ng/ml IL-1β + 25 ng/ml TNFα (medium IL-1β + TNFα) or 25 ng/ml IL-1β + 50 ng/ml TNFα (high IL-1β + TNFα) in basolateral compartments for 48 h. For all studies, the exposure medium was removed, and the apical Caco-2 cell layers were gently rinsed with Hank's balanced salt solution (Gibco BRL) supplemented with 10 mM HEPES (transport medium). After 30 mins incubation in transport medium, 15 nmol mixed primary bile acids in 0.5 mL transport medium was added to the apical compartment. The mixture contained 2.5 nmol CDCA, GCDCA, TCDCA, CA, GCA and TCA each. After 180 mins incubation, the amount of each bile acid was measured in the basolateral compartment using LC/MS/MS.

2.6. Profiling of primary bile acids by LC/MS/MS

Chromatographic separation of primary bile acids was adapted from our previous study (Wang et al., 2022) and carried out using the Shimadzu 8045 System (Kyoto, Japan). Aliquots of samples (1 μL) were separated using Phenomenex 00B-4475-AN (50 mm × 2.1 mm × 1.7 μm × 100 Å, Kinetex C18) with Phenomenex AJ0-8782 as guard column at a column temperature of 40 °C. The mobile phase consisted of MilliQ water with 0.01% formic acid (A) and methanol with 50% acetonitrile (B). The following gradient was used: 0–10 min 30–70% B, 11–19 min 70–98% B and then immediate reduction from 98% to 30% B at 20–25 min with re-equilibration before the next injection. The flow rate was 0.4 mL/min. The electrospray ionization (ESI) was used in negative-ion mode in Liquid Chromatography Mass Spectrometry (LC/MS) analysis (Shimadzu). The optimal ESI source parameters were as follows: nebulizer gas, 3 L/min; heating gas and drying gas, 10 L/min. Interface temperature, desolvation line temperature and heat block temperature were maintained at 300 °C, 250 °C and 400 °C respectively. The multiple reaction monitoring and selective ion monitoring modes were employed for quantification using calibration curves made with commercially available standards. Data were collected and processed by using the LabSolutions software (Shimadzu).

2.7. Cytokine quantification by ELISA following stimulation of cells

THP-1 monocytes were exposed to 50 ng/ml PMA at 1.8×10^5 cells/well in 12-well plate for 24 h. After removing PMA, the differentiated THP-1 macrophages were exposed to LPS (0–1000 ng/ml) for 6 h, or pre-exposed to 0.5% PBS or 10 ng/ml LPS for 2 h followed by 4 h incubation with DON (0, 1.25, 2.5 and 5 μM) in the exposure medium. Medium was collected to quantify the concentrations of IL-1β and TNFα.

After establishing the healthy or inflamed models, the co-cultures were exposed to 0.5% DMSO or DON (0.5 or 2.5 μM) in both apical and basolateral compartments. Medium was collected from basolateral compartment of healthy or inflamed co-cultures at 4 h after starting the exposure to quantify the concentrations of IL-1β and TNFα.

Cell layers of 18 day differentiated Caco-2 monocultures were exposed to DMSO, 10 ng/ml LPS, inflamed THP-1 condition medium, 50 pg/ml IL-1β + 100 pg/ml TNFα (low IL-1β + TNFα) or 25 ng/ml IL-1β + 50 ng/ml TNFα (high IL-1β + TNFα) in basolateral compartment and/or 2.5 μM DON in both apical and basolateral compartment for 4 h. The inflamed THP-1 condition medium was collected at 2 h after 10 ng/ml LPS exposure of THP-1 macrophages. The concentrations of IL-1β, TNFα or IL-8 in the basolateral compartment of Caco-2 cells were quantified by ELISA according to the manufacturer's instructions (Biolegend, San Diego, CA, USA). The Limits of Detection (LOD) for IL-1β, TNFα and IL-8 are 0.5, 2 and 2 pg/ml respectively (Biolegend).

2.8. Statistics

Data are presented as the mean of three biological replicates ± SD, with two technical replicates for the bile acid transport experiments and three for the other experiments. Statistical analysis was performed by using the Student *t* test for comparison of 2 groups or ANOVA followed by the Dunnett test for comparison of 3 or more groups. $p < 0.05$ was considered as statistically significant. If detected values are <LOD, half LOD was used in the statistical evaluation. All data were analysed with SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. DON did not further increase pro-inflammatory cytokines secretion from LPS-pre-stimulated THP-1 macrophages

Macrophages are the most numerous immune cells in the intestinal mucosa, which mediate the pro-inflammatory responses upon exposure to xenobiotics (Ponce de León-Rodríguez et al., 2019). We studied the DON (0–10 μM) induced secretion of pro-inflammatory cytokines IL-1β and TNFα following a 4-hour exposure by naïve or 2 h LPS-pre-stimulated THP-1 macrophages. The used concentrations of DON (0–10 μM) did not induce cytotoxicity in both naïve and LPS-pre-stimulated THP-1 macrophages (Fig. 2A and D). The pro-inflammatory cytokines IL-1β and TNFα have long been used as markers of (intestinal) inflammation, but *in vitro* measurements at timepoints later than 4 h after initial exposure are not relevant because of instability of these cytokines in the cell culture medium (Dinarello, 1997; Schildberger, Rossmanith, Eichhorn, Strassl, & Weber, 2013). Upon DON exposure, the release of IL-1β and TNFα by naïve THP-1 macrophages concentration-dependently increased to 201.3 ± 39.1 and 34.5 ± 2.3 pg/ml respectively, compared to 72.4 ± 24.0 pg/ml and 11.0 ± 1.9 pg/ml respectively without DON exposure (Fig. 1B and C). The release of IL-1β and TNFα in LPS-pre-stimulated THP-1 macrophages was 430.7 ± 146.2 pg/ml and 355.7 ± 27.9 pg/ml respectively without DON exposure. DON exposure did not influence the pro-inflammatory cytokines release in LPS-pre-stimulated THP-1 macrophages (Fig. 2E and F). Control experiments using increasingly high LPS concentrations indicated the capability of THP-1 macrophages to secrete higher amounts of these pro-inflammatory cytokines. The release of IL-1β and TNFα concentration-dependently increased up to 1569.4 ± 1007.0 and 786.8 ± 31.0 pg/ml following 1000 ng/ml LPS exposure (Fig. 2G and H). Together, these data indicate that DON exposure increased the pro-inflammatory cytokines secretion by non-inflamed THP-1 macrophages, while DON exposure did not further increase the pro-inflammatory cytokines secretion by inflamed THP-1 macrophages.

3.2. Creation of in vitro healthy and inflamed co-culture models

To emulate a healthy and inflamed intestinal mucosa *in vitro,* naïve or LPS-pre-stimulated THP-1 macrophages were co-cultured with differentiated Caco-2 cells in Transwells (Fig. 3A). We measured the concentration of the pro-inflammatory cytokines IL-1β and TNFα in the

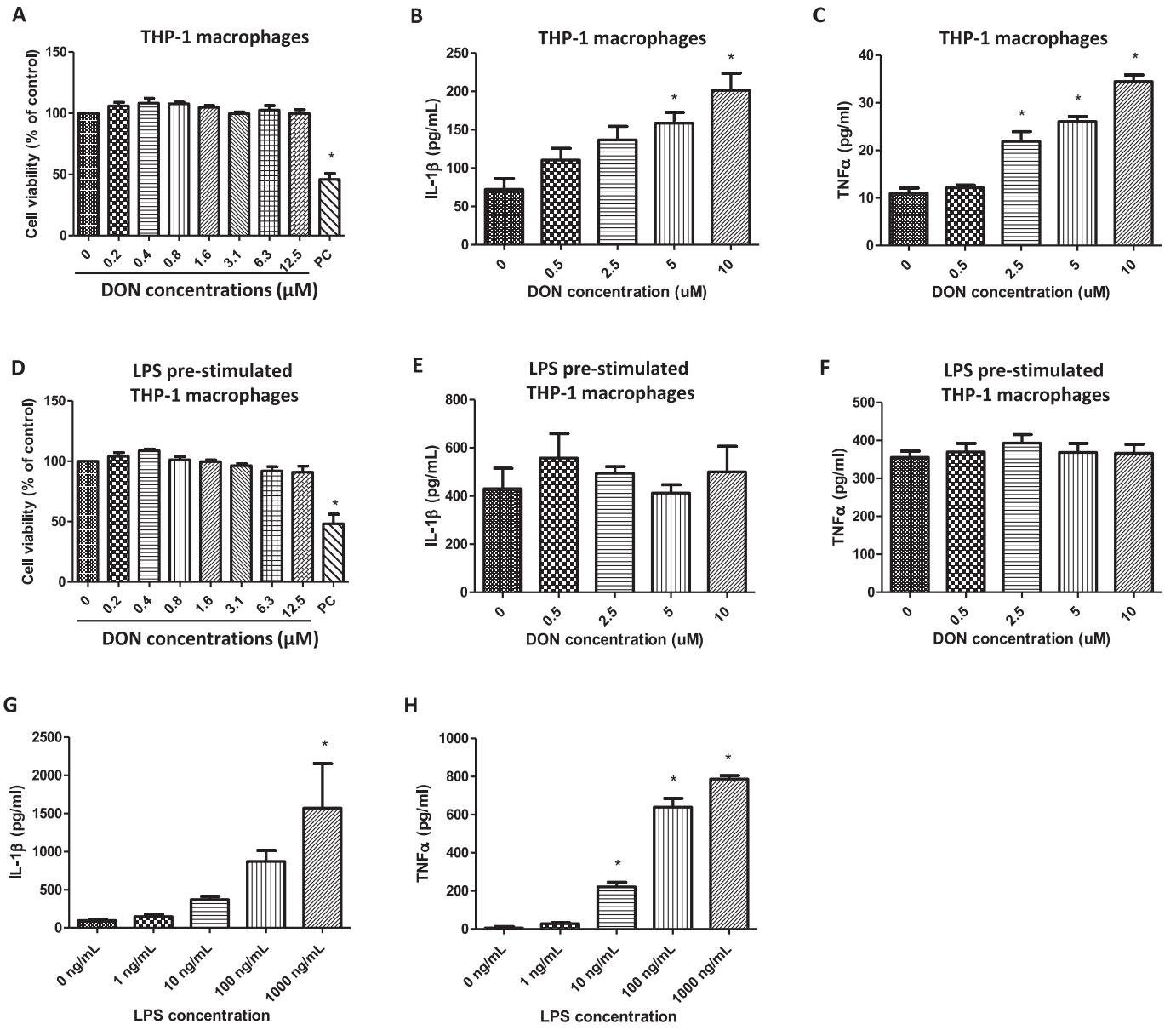

Fig. 2. Pro-inflammatory effects of DON on THP-1 macrophages. (A) The cell viability of naïve THP-1 macrophages was measured using the WST-1 assay following 4 h DON exposure (0–12.5 μM). 0.1% Triton X-100 was used as the positive control. Release of IL-1β (B) and TNFα (C) from naïve THP-1 macrophages following DON exposure (0–10 μM) was measured by ELISA at 4 h. (D) The cell viability of 2 h LPS pre-stimulated THP-1 macrophages was measured using the WST-1 assay following 4 h DON exposure (0–12.5 μM). Release of IL-1β (E) and TNFα (F) from 2 h LPS pre-stimulated THP-1 macrophages following DON exposure (0–10 μM) was measured by ELISA at 4 h. Release of IL-1β (G) and TNFα (H) from THP-1 macrophages following LPS (0–1000 ng/ml) exposure was measured by ELISA at 6 h. Data were expressed as mean ± SD, n = 3. Technical replicates for each experimental setting = 3 *: Mean values significantly differ from solvent control ($p < 0.05$).

basolateral compartment after 4 h of co-culturing (Fig. 3B and C). IL-1β and TNFα concentrations were 77.9 ± 27.6 and 19.6 ± 42.0 pg/ml in the healthy co-cultures, which increased to 1566.7 ± 68.0 pg/ml and 571.6 ± 57.9 pg/ml respectively in inflamed co-cultures.

Co-culturing of inflamed THP-1 macrophages with Caco-2 cells only temporarily affected the barrier properties of the Caco-2 cell layers (Fig. 3D). The TEER values of inflamed co-cultures were significantly decreased to 81.2 ± 3.9% of healthy co-cultures at 4 h of co-culturing and fully recovered to 91.6 ± 6.8% and 98.5 ± 6.9% of healthy co-cultures at 24 and 48 h. The paracellular permeability of the Caco-2 cell layers was studied by fluorescein permeation, which was similar for both healthy and inflamed co-cultures at 48 h of co-culturing (8.7 ± 0.7% and 8.2 ± 1.4% respectively). Based on these results we conclude that the continued exposure to 10 ng/mL LPS in the inflamed co-culture model did not result in prolonged effects on the barrier integrity and paracellular permeability of the Caco-2 cell layers at 48 h of co-culturing.

3.3. *Reduced primary bile acid transport across the Caco-2 cell layer in inflamed co-cultures compared with healthy co-cultures*

The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA), together with their glycine and taurine conjugates (i.e. GCA, GCDCA and TCA, TCDCA respectively), constitute most of the bile acid pool in the human small intestinal lumen (Hofmann, 1977). To assess the transport of these primary bile acids across Caco-2 cell layers (in the co-culture model emulating a healthy or inflamed intestinal mucosa), Caco-2 cell layers were exposed apically to CA, CDCA and their glycine

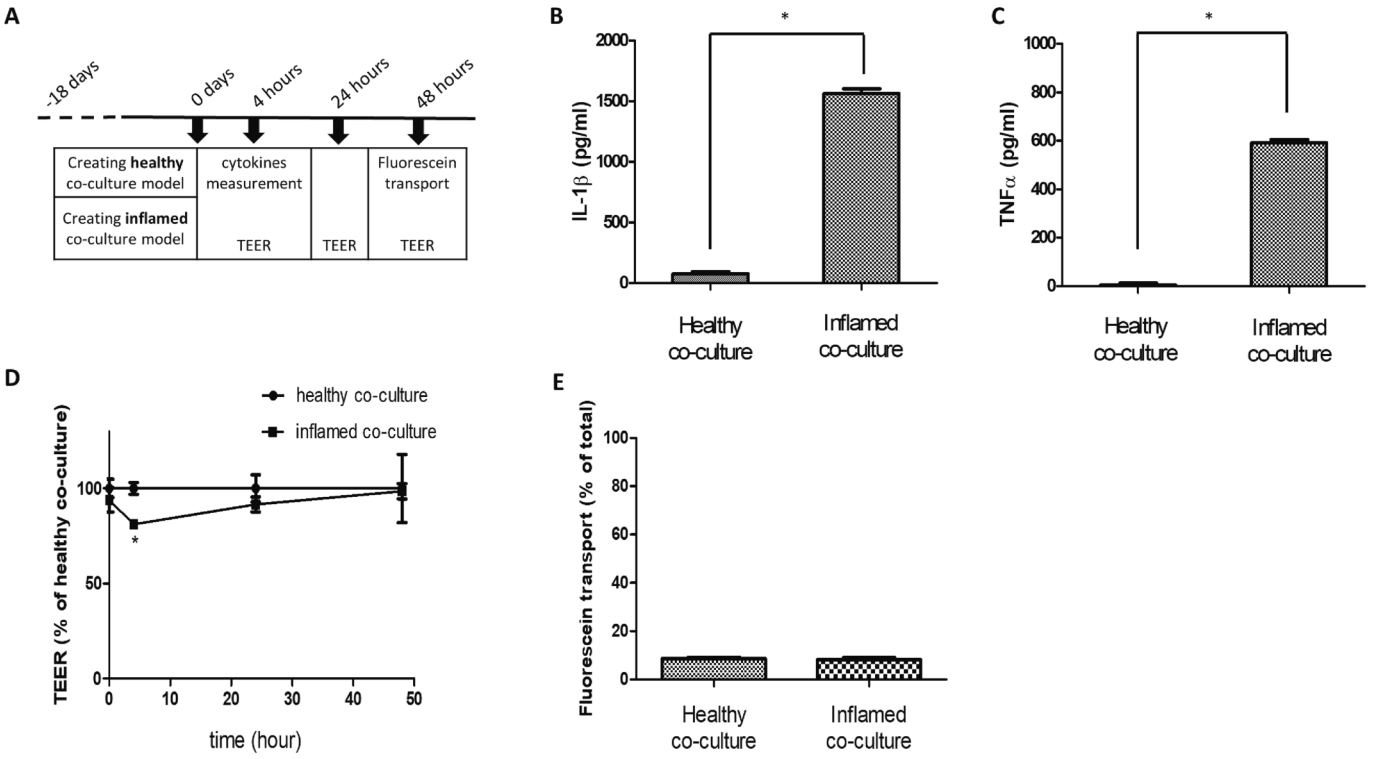

Fig. 3. Characterisation of *in vitro* healthy and inflamed co-culture models. (A). Overview of the experimental time line, for the inflamed co-culture LPS was continuously present in the basolateral compartment. Release of IL-1β (B) and TNFα (C) in the basolateral compartment of the healthy and inflamed co-cultures was measured by ELISA at 4 h. The integrity of Caco-2 cell layer of the healthy and inflamed co-cultures was determined by TEER measurements during 48 h after co-culturing (D). The paracellular permeability was measured by Fluorescein transport at 48 h after co-culture (E). Data were expressed as mean ± SD, n = 3. *: Mean values significantly differ between healthy and inflamed co-cultures ($p < 0.05$).

or taurine conjugates with equal amount at 48 h of co-culturing with healthy and inflamed THP-1 macrophages (Fig. 4A). As shown in Fig. 4B–H, after 180 mins of apical exposure to the six bile acids, the amounts of CA, GCA and TCA in the basolateral compartment of healthy co-cultures were 0.5 ± 0.07, 0.8 ± 0.1 and 0.9 ± 0.2 nmol respectively, which were lower than the amounts of CDCA and its corresponding conjugates GCDCA and TCDCA (1.5 ± 0.6, 2.1 ± 0.5 and 2.4 ± 0.5 nmol respectively). The total amount of primary bile acids in the basolateral compartment of the healthy co-culture after 180 mins transport was 8.3 ± 2.0 nmol whereas the amount in the basolateral compartment of the inflamed co-culture was significantly ($p < 0.05$) lower (4.8 ± 0.5 nmol). In the basolateral compartment of the inflamed co-culture, the amounts of CA, GCA, TCA, GCDCA and TCDCA were 0.3 ± 0.1, 0.3 ± 0.1, 0.3 ± 0.1, 0.9 ± 0.2 and 1.2 ± 0.2 nmol respectively after 180 mins of apical primary bile acid exposure. The amounts of CA, GCA, TCA, GCDCA and TCDCA were significantly lower in the inflamed co-culture compared with those in healthy co-culture. However, the amount of CDCA was similar in the basolateral compartments between healthy and inflamed co-cultures. These results show that for most primary bile acids the transport decreased in inflamed co-cultures compared to healthy co-cultures except for CDCA.

3.4. *DON exposure reduced the primary bile acid transport through Caco-2 cell layers in inflamed co-cultures*

Next, we assessed whether DON exposure further decreased the primary bile acid transport in inflamed co-cultures. Inflamed co-cultures were pre-incubated with DON (0, 0.5 or 2.5 μM) in both the apical and basolateral compartments for 48 h. Then DON was removed, and the inflamed co-cultures were apically exposed to the mixture of primary bile acids (Fig. 5A). After 180 mins incubation to allow for transport, the total amount of primary bile acids in basolateral compartment of inflamed co-cultures without DON pre-exposure was 4.5 ± 0.3 nmol, whereas the bile acid amounts in the basolateral compartment were significantly lower ($p < 0.05$) following 0.5 and 2.5 μM DON pre-exposure (3.2 ± 0.7 and 2.8 ± 0.5 nmol respectively) (Fig. 5B). The amounts of the individual bile acids CA, GCA, TCA, GCDCA and TCDCA (Fig. 5C, E–H) were 0.3 ± 0.03, 0.4 ± 0.1, 0.4 ± 0.1, 1.0 ± 0.2 and 1.1 ± 0.1 nmol respectively in the basolateral compartment of inflamed co-cultures without DON pre-exposure after 180 mins incubations. The amount of these 5 primary bile acids (CA, GCA, TCA, GCDCA and TCDCA) significantly decreased to 0.2 ± 0.02, 0.2 ± 0.02, 0.2 ± 0.04, 0.6 ± 0.02 and 0.7 ± 0.09 nmol respectively following 0.5 μM DON pre-exposure (Fig. 5C, E–H) or 0.1 ± 0.02, 0.2 ± 0.01, 0.2 ± 0.06, 0.5 ± 0.09 and 0.6 ± 0.1 nmol following 2.5 μM DON pre-exposure (Fig. 5C, E–H). The transport of CDCA was not affected by the DON pre-incubations (1.4 ± 0.6, 1.3 ± 0.6 or 1.2 ± 0.3 nmol for 0, 0.5 or 2.5 μM DON pre-exposure respectively) (Fig. 5D). The barrier integrity and paracellular permeability of these inflamed co-cultures was measured before and after primary bile acid transport, and remained stable and the same for all treatment groups (Fig. S2).

3.5. *DON increased the pro-inflammatory cytokines secretion in inflamed co-cultures*

To assess whether DON exposure further increased pro-inflammatory cytokine secretion in inflamed co-cultures, inflamed co-cultures were exposed to DON in both apical and basolateral compartments. The concentration of IL-1β and TNFα concentration-dependently increased to 1590.9 ± 206.9 and 664.9 ± 62.5 pg/ml following 0.5 μM DON exposure or 1812.6 ± 163.7 and 743.9 ± 84.9 pg/ml following 2.5 μM DON exposure (IL-1β and TNFα amounts in inflamed co-cultures without DON exposure were 1402.1 ± 217.7 pg/ml and 590.3 ± 25.6 pg/ml respectively). The increases of IL-1β and TNFα amount reached

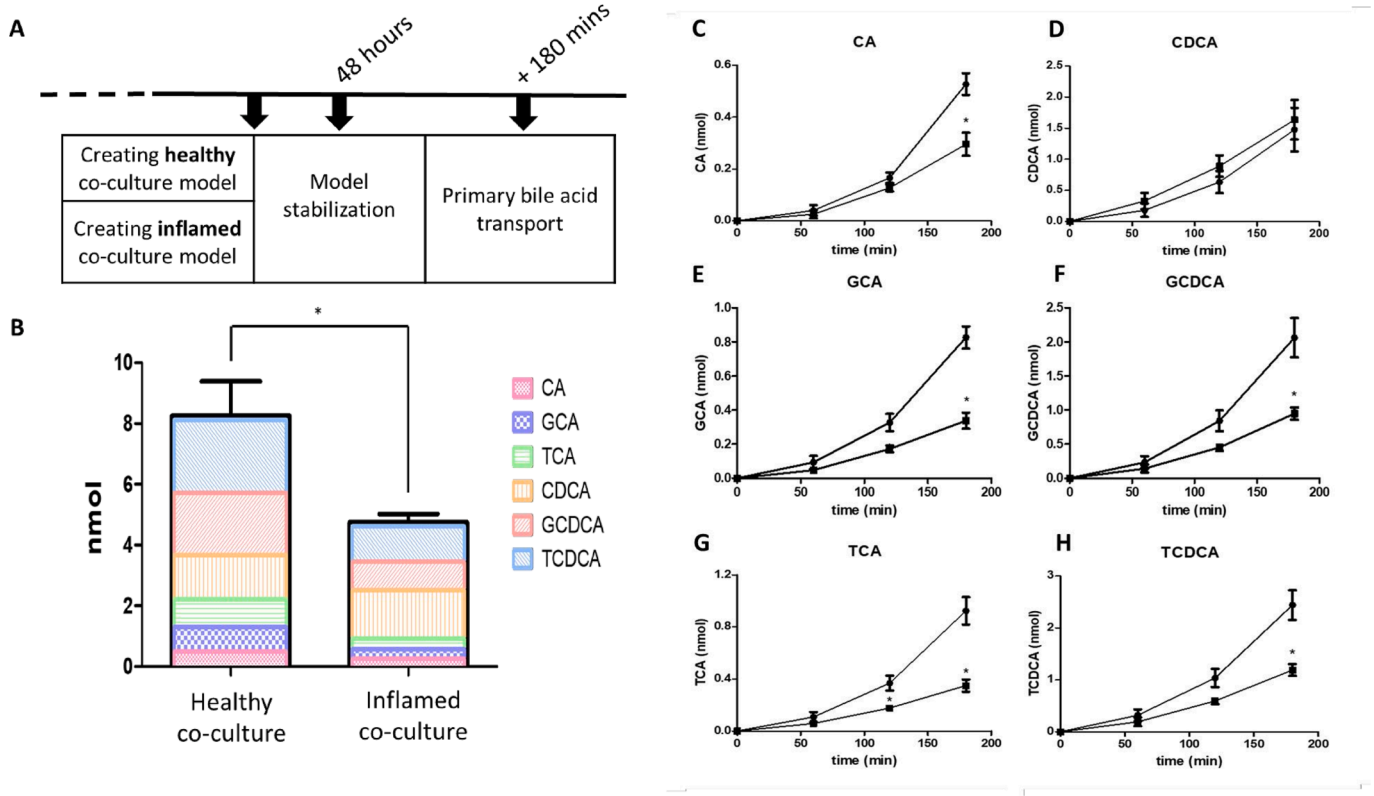

Fig. 4. Reduced primary bile acids transport through the Caco-2 cell layer in inflamed co-cultures compared with healthy co-cultures. After 48 h of co-culturing, healthy and inflamed co-cultures were exposed to 15 nmol mixed primary bile acids in 0.5 mL apical transport medium. The mixture contains 2.5 nmol of each of the following primary bile acids: CDCA, GCDCA, TCDCA, CA, GCA and TCA. (A) Overview of the experimental timeline. (B) The amount of the primary bile acids in the basolateral compartment after 180 mins transport in healthy and inflamed co-cultures. (C–H) The time course of each primary bile acid transport. ●: healthy co-culture, ■: inflamed co-culture. Data were expressed as mean ± SD, n = 3. *: Mean values significantly differ between healthy and inflamed co-cultures ($p < 0.05$).

statistically significant following the 2.5 μM DON exposure in the inflamed co-cultures (Fig. 6A and B). Notably, this increase in pro-inflammatory cytokine secretion was not observed using only the inflamed THP-1 monocultures, in which we observed that DON did not further increase these pro-inflammatory cytokines secretion by inflamed THP-1 macrophages (Fig. 2E and F).

Next, we further explored the interaction of Caco-2 cell layers and inflamed THP-1 macrophages. Firstly, the IL-1β and TNFα secreted in the basolateral compartment of Caco-2 monocultures following 10 ng/ml LPS and/or 2.5 μM DON exposure was below the detection limit (data not shown). It indicates that LPS and/or DON-stimulated Caco-2 monocultures did not directly contribute to the increased IL-1β and TNFα concentration observed in the inflamed co-cultures following DON exposure. IL-8 has long been used as a marker indicating inflamed states of differentiated Caco-2 cells (Van De Walle et al., 2008). The IL-8 secretion in the basolateral compartment of Caco-2 monocultures was below the detection limit following DON and/or LPS exposure. IL-8 secretion from Caco-2 cells which were exposed to the (conditioned) medium of LPS-stimulated THP-1 macrophages was 38.6 ± 3.4 pg/ml, and additional DON co-exposure significantly increased that to 76.9 ± 17.6 pg/ml (Fig. 6C). Together, it indicates that DON does not increase IL-8 secretion from LPS-stimulated Caco-2 cells, but it further increases IL-8 secretion from the Caco-2 cells following co-exposure to the medium of LPS-stimulated THP-1 macrophages.

The conditioned medium contains pro-inflammatory cytokines secreted by THP-1 macrophages following LPS stimulation. Thus, we next co-exposed Caco-2 cells to DON with an IL-1β + TNFα cocktail. DON significantly increased IL-8 secretion from Caco-2 cells following low and high IL-1β + TNFα cocktail exposure to 13.6 ± 5.7 pg/ml and 126.6 ± 36.4 pg/ml respectively, compared to <LOD and 18.6 ± 8.1 pg/ml for low and high IL-1β + TNFα cocktail exposure respectively without a DON exposure. It indicates that the pro-inflammatory cytokines secreted by LPS-stimulated THP-1 macrophages could reinforce the direct proinflammatory effect of DON on Caco-2 cells.

3.6. *Pro-inflammatory cytokines decreased primary bile acid transport through Caco-2 cell layers*

To assess whether pro-inflammatory cytokines exposure that represents the DON induced cytokine secretion in the co-culture model, would decrease primary bile acid transport across Caco-2 cell layers, Caco-2 cell layers were preincubated with IL-1β + TNFα in the basolateral compartments for 48 h. Then the Caco-2 monocultures were apically exposed to the mixture of primary bile acids. After 180 mins incubation, the total amount of primary bile acids in basolateral compartment of Caco-2 cell layers without IL-1β + TNFα pre-exposure was 9.0 ± 0.2 nmol. A reduction of primary bile acid transport was found in Caco-2 cell layers pre-exposed to the pro-inflammatory cytokines (8.7 ± 0.4 nmol, 7.1 ± 0.4 nmol and 6.2 ± 0.1 nmol for low, medium and high IL-1β + TNFα pre-exposure respectively) (Fig. 7A). The amounts of the individual bile acids CA, GCA, TCA, GCDCA and TCDCA (Fig. 7B, D–G) were 0.8 ± 0.1, 1.0 ± 0.2, 1.0 ± 0.01, 2.1 ± 0.1 and 1.8 ± 0.1 nmol respectively in the basolateral compartment of Caco-2 cell layers without pro-inflammatory cytokines pre-exposure. The amount of these 5 primary bile acids (CA, GCA, TCA, GCDCA and TCDCA) significantly decreased to 0.6 ± 0.1, 0.5 ± 0.04, 0.5 ± 0.06, 1.3 ± 0.1 and 1.1 ± 0.03 following high IL-1β + TNFα pre-exposure. The transport of CDCA was not affected by the pro-inflammatory cytokines pre-incubations (2.3 ± 0.2, 2.1 ± 0.2 2.2 ± 0.1 or 2.2 ± 0.1 nmol for solvent, low, medium and high IL-1β + TNFα pre-exposure respectively)

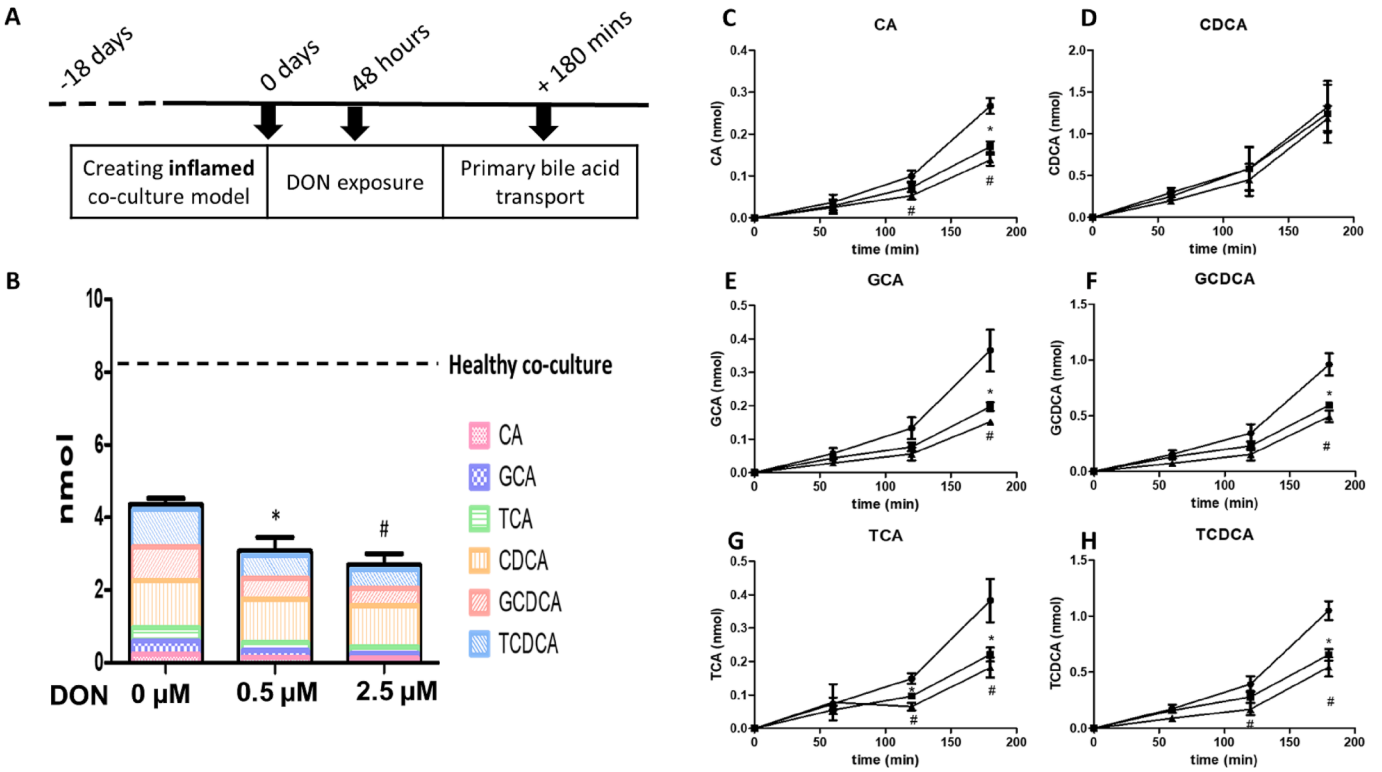

Fig. 5. DON decreased the primary bile acid transport across Caco-2 cell layers in inflamed co-cultures. Inflamed co-cultures were pre-incubated with DON (0, 0.5 or 2.5 μM) in both apical and basolateral compartments for 48 h before exposure to 15 nmol mixed primary bile acids in 0.5 mL apical transport medium. The mixture contains 2.5 nmol of each of the following primary bile acids: CDCA, GCDCA, TCDCA, CA, GCA and TCA. (A). Overview of the experimental time line. (B) The amount of the primary bile acids in the basolateral compartment after 180 mins transport in inflamed co-cultures with or without DON pre-exposure. (C–H) The time course of each primary bile acid transport.●: inflamed co-cultures without DON pre-exposure, ■: inflamed co-culture with 0.5 μM DON pre-exposure, ▲: inflamed co-culture with 2.5 μM DON pre-exposure. Data were expressed as mean ± SD, n = 3. *: Mean values significantly differ between 0 and 0.5 μM DON pre-exposed inflamed co-cultures ($p < 0.05$). #: Mean values significantly differ between 0 and 2.5 μM DON pre-exposed inflamed co-cultures ($p < 0.05$).

(Fig. 7C). The paracellular permeability of the Caco-2 cell layers was same for all treatment groups measured by Fluorescein transport assay after primary bile acid transport (Fig. S3).

4. Discussion

In this study, we aimed to explore the effects of DON exposure on primary bile acid transport in an inflamed intestinal mucosa. Previously we have shown that DON reduced bile acid transport across Caco-2 cell layers (Wang et al., 2022). This reduced transport may associated with the increased levels of pro-inflammatory cytokines IL-1β and TNFα following DON exposure (Chen et al., 2002). Here we showed that DON cannot further increase the secretion of IL-1β and TNFα by LPS-inflamed THP-1 macrophages. In the intestinal mucosa, epithelial cells and tissue residing macrophages communicate with each other. To study the paracrine interaction we first established an *in vitro* co-culture model for the inflamed human intestinal mucosa, using differentiated Caco-2 cell layers and LPS-pre-stimulated THP-1 macrophages grown in Transwells. The inflamed co-cultures released significantly higher amounts of IL-1β and TNFα (compared to the healthy co-culture models) which did not result in prolonged effects on the barrier integrity and paracellular permeability of the Caco-2 cell layers in the co-culture model. A decreased transport of most primary bile acids (except for CDCA) was observed across the Caco-2 layers of the inflamed co-cultures compared to healthy co-cultures. The transport of these primary bile acids was further reduced following DON exposure. In addition, exposure to DON increased pro-inflammatory cytokines IL-1β and TNFα secretion in inflamed co-cultures. IL-1β and TNFα secreted by the LPS-stimulated THP-1 macrophages reinforce the direct pro-inflammatory effect of DON on Caco-2 cells. Exposure of Caco-2-cell layers to IL-1β and TNFα that represents the DON-induced cytokine profile reduced the primary bile acid transport across Caco-2 cell layers.

Human exposure to deoxynivalenol (DON) is associated with intestinal inflammation (EFSA, 2017; Van De Walle et al., 2008). Our results showed that DON concentration-dependently (0–10 μM) increased the secretion of pro-inflammatory cytokines IL-1β and TNFα in naïve THP-1 macrophages. Similarly, DON concentration-dependently upregulated the gene expression and secretion of pro-inflammatory cytokines TNFα, IL-6 and IL-12 in RAW 264.7 murine macrophages (Molagoda, Choi, Jin, Lee, & Kim, 2019). Transcriptome studies confirmed that DON exposure induced a robust expression of inflammatory response genes including cytokines, cytokine receptors and transcription factors in RAW 264.7 cells (He, Pan, Zhou, & Pestka, 2013). Increased IL-1β, IL-6 and IL-8 gene expression was also found in human PBMCs following DON exposure (Islam et al., 2006). DON exerts its immunostimulant effect on immune cells by activating MAPK pathways via ribotoxic stress (Pestka, Zhou, Moon, & Chung, 2004). However, our results showed that DON (0–10 μM) did not further increase the pro-inflammatory cytokines IL-1β and TNFα secretion from LPS-pre-stimulated THP-1 macrophages. In line with this, it was previously shown that DON decreased pro-inflammatory IFNβ reporter activity and resulted in pro-inflammatory mediator nitric oxide (NO) overproduction in LPS-stimulated RAW264 cells (Sugiyama, Muroi, Tanamoto, Nishijima, & Sugita-Konishi, 2010), inhibited pro-inflammatory signal pathway NF-κB activity in these cells and in THP-1 cells (Sugiyama et al., 2016), and decreased the TNFα and IL-6 gene expression in LPS-stimulated lung macrophages (Liu et al., 2020). This can be explained by the interaction of LPS and DON in creating a cellular immune signalling response. LPS stimulates TLR4, which induces the inflammatory cytokine production through the TLR4/NF-κB pathways (Piccinini, Zuliani-Alvarez, Lim, & Midwood, 2016).

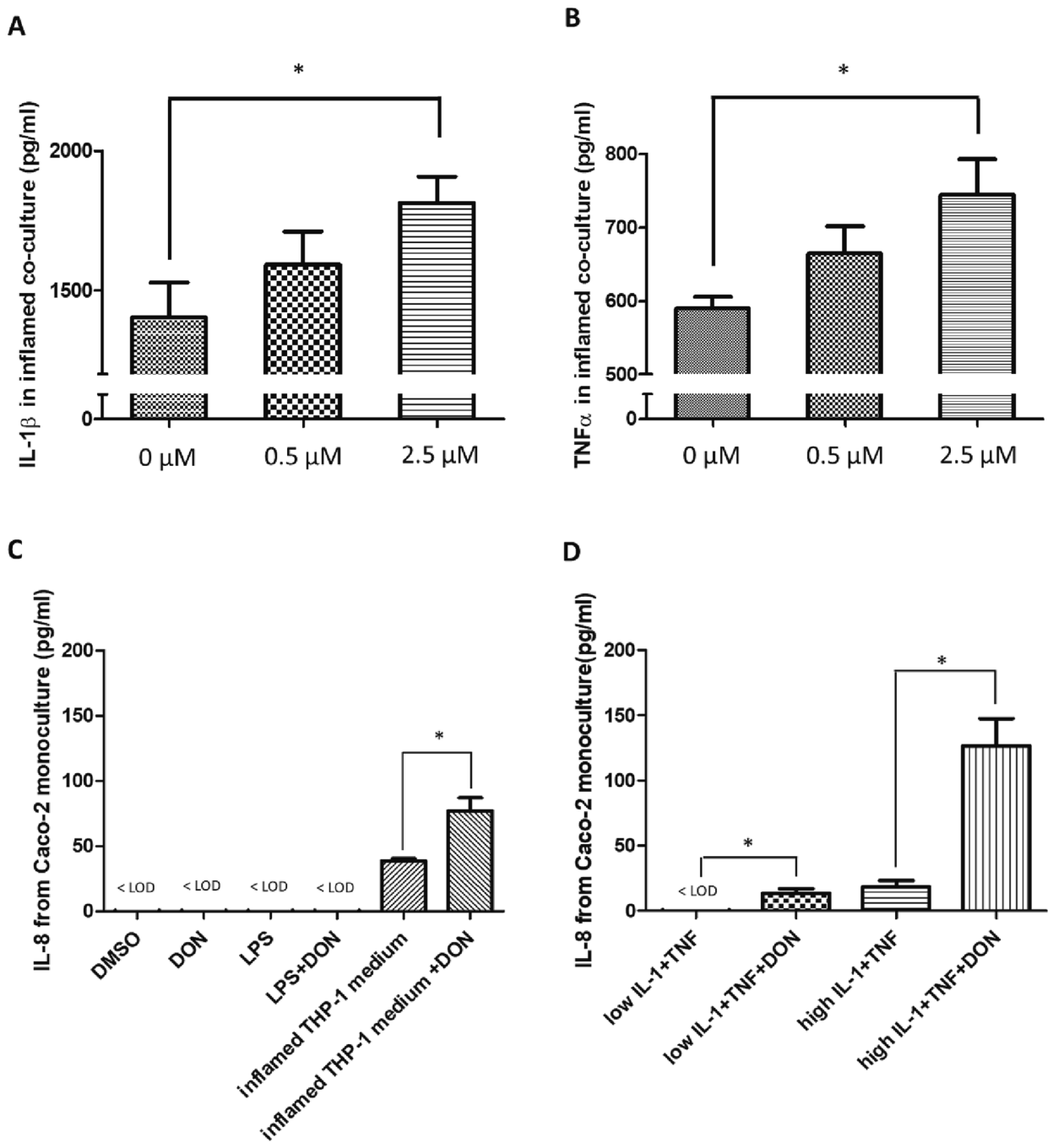

Fig. 6. DON exposure increased pro-inflammatory cytokines secretion in inflamed cultures. Release of IL-1β (A) and TNFα (B) in the basolateral compartment of the inflamed co-cultures following DON (0, 0.5 or 2.5 μM) exposure in both apical and basolateral compartments at 4 h. The IL-8 secretion from basolateral compartment of Caco-2 cells following DMSO, 10 ng/ml LPS, inflamed THP-1 condition medium exposure in basolateral compartment and/or 2.5 μM DON in both apical and basolateral compartments at 4 h. The inflamed THP-1 condition medium was collected at 2 h after 10 ng/ml LPS exposure in THP-1 macrophages. The differences in IL-8 concentrations between Caco-2 exposed to conditioned medium of inflamed THP-1 that have (or not) been exposed to DON is shown (C). The IL-8 secretion from Caco-2 cells following 50 pg/ml IL-1β + 100 pg/ml TNFα (low IL-1β + TNFα) or 25 ng/ml IL-1β + 50 ng/ml TNFα (high IL-1β + TNFα) exposure in basolateral compartment and 2.5 μM DON in both apical and basolateral compartments at 4 h (D). Data were expressed as mean ± SD, n = 3. *: Mean values significantly differ the indicated groups ($p < 0.05$). LOD: Limit of Detection (see materials and methods).

The activation of TLR4 is mediated by myeloid differentiation factor 88 (MyD88) (Laird et al., 2009). DON exposure decreases the expression of MyD88 in immune cells (Sugiyama et al., 2016). By this way, DON inhibits the activation of TLR4/NF-κB pathways and limits the secretion of the pro-inflammatory cytokines in LPS-stimulated THP-1 macrophages. Paracrine interaction between the intestinal epithelial and intestinal immune cells, mainly by cytokines, plays an important role in the regulation of intestinal inflammation (Bamias & Cominelli, 2016). The inflammatory status of the intestinal epitheliums affects the primary bile acid intestinal transport (Chen et al., 2002). Therefore, we next studied if DON modifies pro-inflammatory cytokine signalling and if DON affects the intestinal primary bile acid transport using an *in vitro* co-culture model of the in inflamed intestine.

In our study, 18-days differentiated Caco-2 cell layers grown on Transwells membranes were used to represent the intestinal epithelial barrier (Meunier, Bourrie, Berger, & Fabre, 1995). PMA-differentiated

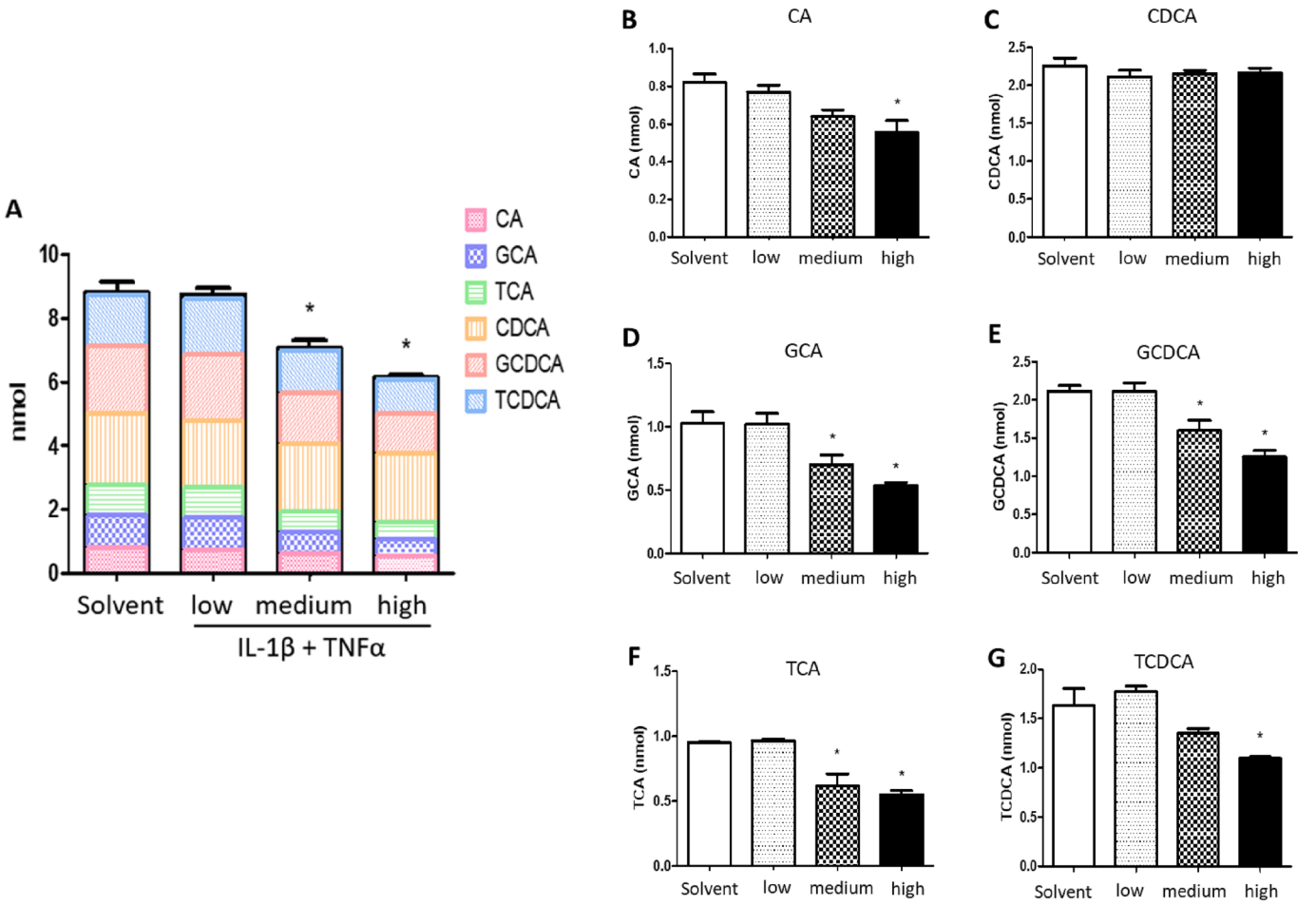

Fig. 7. Pro-inflammatory cytokines decreased the primary bile acid transport across Caco-2 cell layers. Caco-2 cell layers were pre-incubated with 2.5 ng/ml IL-1β + 5 ng/ml TNFα (low IL-1β + TNFα), 10 ng/ml IL-1β + 25 ng/ml TNFα (medium IL-1β + TNFα) or 25 ng/ml IL-1β + 50 ng/ml TNFα (high IL-1β + TNFα) in basolateral compartments for 48 h before exposure to 15 nmol mix of primary bile acids in 0.5 mL apical transport medium. The mixture contains 2.5 nmol of each of the following primary bile acids: CDCA, GCDCA, TCDCA, CA, GCA and TCA. (A) The total amount of the primary bile acids in the basolateral compartment after 180 mins transport. (B–G) The amount of each primary bile acids in the basolateral compartment after 180 mins transport. Data were expressed as mean ± SD, n = 3. *: Mean values significantly differ between solvent and the indicated groups ($p < 0.05$).

THP-1 macrophages were grown in the basolateral compartments of Transwells and were used to represent the immune cells in intestinal lamina propria (Ponce de León-Rodríguez et al., 2019). The release of pro-inflammatory cytokines IL-1β and TNFα was higher in the basolateral compartments of inflamed co-cultures compared with that in healthy co-cultures at 4 h after co-culturing. This indicates that the stimulation of the THP-1 macrophages with LPS emulates inflammatory intestinal conditions *in vitro* as shown before (Kämpfer et al., 2017). We aimed to establish a controlled inflammation model with the same paracellular permeability as a healthy model for transport studies. For this, we monitored the Caco-2 barrier integrity of healthy and inflamed co-cultures over 48 h. Our results showed a sudden decrease of TEER in inflamed co-cultures at 4 h compared with the healthy co-cultures, while this difference could not be observed at 48 h after co-culturing. This confirms previous observations in a very comparable co-culture model using Caco-2 and THP-1 cells (Kämpfer et al., 2017). Pro-inflammatory cytokines like IL-1β and TNFα have been shown to decrease the tight junction expression thereby increase the paracellular permeability in Caco-2 cell layers (Capaldo & Nusrat, 2009). Caco-2 cell layers are able to re-establish barriers after disruption (Lieberth et al., 2021). Thus, the 48 h of co-culturing was set as the starting point for the bile acid transport studies.

We assessed the transport of mixed primary bile acids across Caco-2 cell layers in healthy and inflamed co-cultures. The mixture contained equal amounts of the individual primary bile acids. After 180 mins of apical incubation, in the healthy co-cultures more TCDCA, GCDCA and CDCA is transported to the basolateral compartment compared to CA, GCA and TCA. Most of the bile acids are transported across the intestinal epithelium by the apical transporter ASBT and basolateral transporter OSTα/β (Dawson, Lan, & Rao, 2009). The apical transporter ASBT initiates the transport and is important in controlling the transport rate and has a higher affinity for TCDCA and GCDCA compared to CA, GCA, TCA. The ASBT affinity as indicated by the apparent Michaelis constants (K_m) for CA, GCA, TCA, GCDCA and TCDCA is 15.1, 11.0, 4.39, 0.662 and <0.1 μM respectively (Balakrishnan, Wring, & Polli, 2006). A low K_m value indicates a high affinity of the transporters. The difference of ASBT affinity for individual bile acids may explain the differently transported amounts of TCA, GCA and CA compared with the other bile acids as observed by us. CDCA passes the intestinal epithelial barrier passively, and therefore has no K_m (Balakrishnan et al., 2006). The basolateral transporter OSTα/β has a low affinity but a high capacity for transporting primary bile acids. The K_m value for CA, GCA, TCA, CDCA, GCDCA and TCDCA is >400, >1000, >10000, 23.0, 864.2 and 723.7 μM respectively (Suga, Yamaguchi, Ogura, & Mano, 2019). Thus, the expression of OSTα/β is considered to have less influence of the overall intestinal epithelial transport rate than ASBT following exposure to physiological concentrations of bile acids.

In inflamed co-cultures, the transport of most of the primary bile

acids (CA, GCA, TCA, GCDCA and TCDCA) was decreased except for CDCA if compared with the transport across the healthy co-culture intestinal barrier. Animal studies based on murine, canine and rabbit models of intestinal inflammation displayed decreased primary bile acids reabsorption and increased fecal primary bile acids concentrations (Giaretta et al., 2018; Hou et al., 2018; Sundaram, Wisel, Stengelin, Kramer, & Rajendran, 1998). Bile acid malabsorption is a common symptom of IBD, for this reason bile acids have become an emerging biomarker for IBD clinical diagnosis (Fitzpatrick & Jenabzadeh, 2020). DON exposure further decreased most of the primary bile acids transport across Caco-2 cell layers in inflamed co-cultures, except for CDCA. DON also reduces the transport of (conjugated) bile acids in healthy Caco-2 monolayers (Wang et al., 2022), but in the inflamed-coculture, without and with DON even less bile acids are transported. The barrier integrity and paracellular permeability of these inflamed co-cultures were unaffected upon DON exposure. The decreased transport of these primary bile acids in inflamed co-cultures, as well as the further reduction following DON exposure, was caused by reduced active transport (Wang et al., 2022). The passive diffusion of CDCA transport was not affected in inflamed co-cultures or by DON exposure (Balakrishnan et al., 2006).

The pro-inflammatory cytokines IL-1β and TNFα inhibit the mRNA expression of bile acid active transporter ASBT in Caco-2 and IEC-6 cells (Chen et al., 2002). We found that basolateral exposure of Caco-2 cell layers to IL-1β and TNFα decreased the primary bile acids (CA, GCA, TCA, GCDCA and TCDCA) transport. The passive transport of CDCA was not affected by to IL-1β and TNFα exposure. Clinical observations in human revealed that ASBT gene expression was significantly lower in inflamed regions of individuals with IBD (Jahnel et al., 2014). The decreased active transport of primary bile acids can be explained as the reduction of the expression of ASBT transporter following IL-1β and TNFα exposure (Jahnel et al., 2014). Considering the capability of DON to induce pro-inflammatory cytokine production, it's postulated that the effect of DON on bile acid malabsorption may be mediated by pro-inflammatory cytokines. To validate this hypothesis, we blocked the inflammatory cascade by using MAPK inhibitors and concluded that pro-inflammatory cytokines mediated the reduction in ASBT mRNA expression caused by DON (Wang, Sijs, Bakker, de Haan, & Bouwmeester, 2023).

Using the inflamed THP-1 macrophage and Caco-2 cell layer co-culture model we recapitulated that the pro-inflammatory cytokines IL-1β and TNFα induced reduced transport of primary bile acids (except CDCA). This cannot be explained by a direct effect of DON on inflamed THP-1 cells, as this treatment did not affect the proinflammatory cytokine levels of inflamed THP-1 cells. Nor only a direct effect of DON on the Caco-2 cell layers as the IL-1β, TNFα and IL-8 secretion from DON and/or LPS-stimulated Caco-2 cells was below the limit of detection. This confirms previous findings that exposure to 1.7 μM DON did not increase the IL-8 secretion from LPS-stimulated Caco-2 cells (Van De Walle et al., 2008). Thus, the increased IL-1β and TNFα secretion in inflamed co-cultures following DON exposure was not because of the directly contribution from the LPS-stimulated Caco-2 cells nor of a direct contribution of THP-1 macrophages.

Our results show that DON further increased the pro-inflammatory cytokine IL-8 secretion from Caco-2 cells which were exposed to the medium from LPS-stimulated THP-1 macrophages. In addition, the IL-8 secretion from IL-1β and TNFα stimulated Caco-2 cells was also increased following DON exposure. Similar synergistic effect was observed between DON and IL-1β, as others showed that DON exacerbated the IL-8 gene transcription and IL-8 secretion in IL-1β stimulated differentiated Caco-2 cells (Beisl et al., 2020). IL-1β and other pro-inflammatory cytokines induce pro-inflammatory responses by binding with their receptors and activate the NF-kB via a non-MyD88-dependent pathway, which is not suppressed by DON exposure. Although DON did not increase the IL-1β and TNFα secretion from LPS-pre-stimulated THP-1 macrophages nor from LPS-stimulated Caco-2 cells, the secreted IL-1β and TNFα from the THP-1 macrophages activate Caco-2 cells, which reinforces the direct pro-inflammatory effect of DON on Caco-2 cells and promote IL-8 secretion. The inflamed Caco-2 cells further activate the LPS-pre-stimulated THP-1 macrophages via various factors such as pro-inflammatory interleukins including IL-8, prostaglandin E2 and NO (Al-Ghadban, Kaissi, Homaidan, Naim, & El-Sabban, 2016; Manabe, Kanzato, & Shimizu, 2002; Van De Walle, Hendrickx, Romier, Larondelle, & Schneider, 2010; Ye et al., 2022). This cross-talk between inflamed Caco-2 and LPS-pre-stimulated THP-1 cells leads to the production of more pro-inflammatory cytokines including IL-1β and TNFα and create a cycle in which Caco-2 and THP-1 macrophages potentate each other upon DON exposure, increasing pro-inflammatory cytokines secretion in inflamed co-cultures following DON exposure.

In conclusion, this study revealed that DON further increases IL-1β and TNFα secretion and further decreases the transport of the primary bile acids through Caco-2 cell layers co-cultured with LPS-pre-stimulated THP-1 macrophages, except CDCA which transported by passive diffusion. Although DON did not increase IL-1β and TNFα secretion from LPS-pre-stimulated THP-1 macrophages, the secreted IL-1β and TNFα from the LPS-pre-stimulated THP-1 macrophages activates Caco-2 cells, which reinforces the direct pro-inflammatory effect of DON on Caco-2 cells. IL-1β and TNFα reduced primary bile acid transport across the cell layers of Caco-2 monocultures. Together, this study reveals that the epithelial and immune cell-mediated inflammations potentate each other upon DON exposure. While DON reduces bile acid transport in a healthy intestinal models, the interaction further affects the bile acid intestinal kinetics in an inflamed intestine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Author contributions

JW, LH, and HB contributed to the study conception and design. WB contributed to optimize the LC–MS method for the quantification bile acids. JW conducted all the experimental work and wrote the original draft. Writing, review and editing of the manuscript was done by JW and HB

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113323.

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