Grape seed extract (GSE) modulates *campylobacter* pro-inflammatory response in human intestinal epithelial cell lines

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**ABSTRACT**

The main aim of this study was to investigate the effect of Grape seed extract (GSE) on *Campylobacter* induced-cytokine production by human intestinal cell lines. With this purpose, Caco-2 and HT-29 cells were challenged with culture supernatants from several strains of *C. jejuni* and levels of secreted IL-6, IL-8, and MCP-1 were measured. Cytokine production was higher in HT-29 than in Caco-2 cells, showing different levels of secretion depending of the epithelial cells origin. Also, infective isolates coming from campylobacteriosis patients rendered higher levels of pro-inflammatory cytokines. Co-treatment and pre-treatment of HT-29 cells with GSE and *C. jejuni* significantly reduced cytokines production in a dose-dependent manner. These results make this natural product a putative nutritional tool for use in the treatment of campylobacteriosis, which could contribute to improve disease prevention or reduce disease severity.

**1. Introduction**

*Campylobacter* is the leading cause of bacterial food-borne gastroenteritis worldwide and the species *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) cause more than 95% of the infections attributed to this genus (Ganan, Silván, Carrascosa, & Martínez-Rodríguez, 2012). *Campylobacter* infections in humans can have mild to severe symptoms, which commonly include gastrointestinal manifestations such as self-limiting diarrhea, abdominal cramps, nausea, and fever, but severe neurological sequelae, bacteremia, and other extra intestinal complications may develop less frequently. A hallmark of this human disorder caused by *Campylobacter* is the inflammation of the intestinal mucosa. This pathogen is able to initiate inflammatory signals by interaction (adhesion and/or invasion) with host cells and activate signaling pathways that induce the production of inflammatory cytokines and recruitment of phagocytes, especially neutrophils, into infected tissue (Everest, 2005). Although most infections are resolved without specific treatment, antimicrobial therapy can be critical in invasive or severe infections (Allos, 2001). Even though the virulence attributes of a specific *Campylobacter* strain can determine its ability to infect humans, it is known that immunocompetent people usually
resolve the infection, indicating a key role of immune system in clearing the infection (Iovine, 2008).

Human intestinal epithelial cells are the first site of contact between the host and enteric pathogenic bacteria, stimulating an innate immune response after detection of an infectious process. One of the most significant is the rearrangement of gene expression leading to the production of pro-inflammatory cytokines (Hanada & Yoshimura, 2002; Hoffmann, Dittrich-Breiholz, Holtmann, & Kracht, 2002; Jenner & Young, 2005). Among them, interleukin-6 (IL-6), interleukin-8 (IL-8), and the monocyte chemoattractant protein 1 (MCP-1), are chemokines produced by epithelial cells. They have potent chemoattractant properties for lymphocytes and neutrophils into infected tissue (Stadnyk, 2002). Some Campylobacter strains have been reported to induce the secretion of IL-6 (Bahrami, Macfarlane, & Macfarlane, 2011; Jones, Tötemeyer, Maskell, Bryant, & Barrow, 2003), IL-8 (Borrmann, Berndt, Hänel, & Köhler, 2007; Li et al., 2011; MacCallum, Harris, Haddock, & Everest, 2006), and MCP-1 (Hu & Hickey, 2005) in human intestinal epithelial cells. Although this immune activity is essential to the development of an appropriate response to infection, an exacerbate immune response could induce tissue damage (Hu & Kopecko, 2008). For this reason, it would be important to modulate the immune response avoiding a tissue damage which could trigger a chronic inflammatory bowel disease.

Grape seed extracts (GSE) are particularly rich in bioactive substances such as phenolic compounds. The seeds contain 60–70% of the extractable polyphenols in grapes. The phenolic content of seeds may range from 5% to 8% by weight. The most abundant phenolic compounds are flavonoids, essentially catechins (catechin, epicatechin, and procyanidins) and their polymers (Badet, 2011; Silvan et al., 2013). GSE has been marketed as a dietary supplement due to its beneficial effects and free radical-scavenging ability (Mandic, Dilas, Cetkovic, Canadanovic-Brunet, & Tumbas, 2008). Recently, we have demonstrated the usefulness of GSE as antimicrobial against Campylobacter and the role of phenolic compounds in the antimicrobial effect (Silvan et al., 2013). Also, GSE have shown other pharmacological activities, including anti-inflammatory activity (Bibi, Kang, Yang, & Zhu, 2016; Yang, Wang, Kanga, & Zhu, 2014). However, its effect on the cytokines secretion from intestinal epithelial cells induced by Campylobacter is unknown.

In the present work the ability of C. jejuni to stimulate the production of IL-6, IL-8, and MCP-1, and the feasibility of GSE as a modulating agent in the induction of pro-inflammatory cytokines in intestinal epithelial cells by C. jejuni has been evaluated.

2. Material and methods

2.1. Materials

Dulbecco’s Modified Eagle’s Medium (DMEM), non-essential amino acids (NEAA), phosphate buffered saline (PBS), penicillin/streptomycin (5000 U/mL), and trypsin/EDTA solution (170,000 U/L) were purchased from Lonza (Cultek S.L.U., Madrid, Spain). Fetal bovine serum (FBS) (South American origin, Hyclone) was obtained from Thermo Scientific (Cultek). Cell culture plastic materials were obtained from Sarstedt (Nümbrecht, Germany). Enzyme-linked immunosorbent assays (ELISA) kits for human IL-6, IL-8, and MCP-1 detection were purchased from Diaclone (Besancon, France). Standards used for high
performance liquid chromatography (HPLC) analysis and phenolic quantification were purchased from Extrasynthese (Lyon, France).

2.2. Grape seed extract (GSE) preparation

Grape seed powder (Laboratorios GSN, Madrid, Spain) was purchased from a local market. The powdered grape seeds (500 mg) was dissolved in 10 mL of distilled water and stirred for 5 min at room temperature. The suspension obtained was centrifuged at 4000 rpm for 10 min. The supernatant was collected and sterilized by filtration with a pore size membrane of 0.22 μm (Sarstedt). The GSE obtained was stored at −20°C until use.

2.3. Bacterial strains, growth media, and culture conditions

Bacteria used in this study included 6 different strains of C. jejuni. Strain specification and origin of the specimen is provided in Table 1. All strains were stored at −80°C. The agar plating medium consisted of Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (MHB) (Becton, Dickinson & Company, New Jersey, USA). Liquid growth medium consisted of Brucella Broth (BB) (Becton, Dickinson, & Company). The frozen strains were reactivated by inoculation in MHB and incubation under microaerophilic conditions (85% N2, 10% CO2, 5% O2) using a Variable Atmosphere Incubator (VAIN) (MACS-VA500, Don Whitley Scientific, Shipley, UK) at 42°C for 48 h. Isolated colonies were inoculated into 50 mL of BB and incubated under stirring at 130 rpm on an orbital shaker at 42°C for 24 h in microaerophilic conditions in the VAIN. For preparation of infective inoculum, 5 mL of bacterial cultures were centrifuged for 10 min at 5000 rpm, the supernatants were discarded and the pellets were resuspended in 5 mL of invasion medium consisted in DMEM without antibiotics. These bacterial inoculum cultures (∼1 × 10⁸ colony forming unit (CFU)/mL) were used for the infection of intestinal human cell lines. The amount of bacteria added was standardized between experiments by measuring the CFU/mL of the bacterial suspension.

2.4. Cell cultures

For experimental procedures human colonic epithelial cells, Caco-2 and HT-29, were used. Cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM supplemented with 10% FBS, 1% NEAA and 1% penicillin/streptomycin. Cells were plated at densities 1 × 10⁵ cells in 75 cm² tissue culture flasks and maintained at 37°C under 5% CO2 in a humidifier atmosphere. The culture medium was changed every two days. Confluent stock cultures were trypsinized (Trypsin/EDTA) and cells were seeded in 24-well plates (∼5 × 10⁴ cells/well) and

| Specie     | Strain | Origin     | Source                        |
|------------|--------|------------|-------------------------------|
| C. jejuni  | LP1    | Clinical   | Hospital La Paz, Madrid       |
|            | 118    | Clinical   | Hospital Carlos III, Madrid   |
|            | CIII   | Clinical   | Hospital Carlos III, Madrid   |
|            | CN1    | Veterinary | CIAL⁵                        |
|            | CN2    | Veterinary | CIAL⁵                        |
|            | CIAL1  | Veterinary | CIAL⁵                        |

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Table 1. Source of C. jejuni strains.
incubated in culture medium at 37°C under 5% CO₂ in a humidifier incubator until a monolayer was formed. The culture medium was changed every two days. All experiments were carried out between passage 10 to passage 30 to ensure cell uniformity and reproducibility.

2.5. Infection of human intestinal cell lines

Human intestinal epithelial cell monolayers (Caco-2 and HT-29) were washed three times with PBS to eliminate the antibiotics, infected with 500 μL of bacterial suspensions (∼10⁸ CFU/mL), and co-treated or pre-treated with GSE at different concentrations in the experimental conditions summarized in Table 2. The infected monolayers were incubated at 37°C in a 5% CO₂ humidified atmosphere to allow the bacteria to adhere and invade the cells. Uninfected cells were included in the experiment as a control. At the end of each time point the cell supernatants were collected into Eppendorf tubes, particulate material was removed by centrifugation for 20 min at 13,000 rpm and samples were stored at −20°C until analysis were performed. All experiments were carried out in triplicate.

2.6. Cytokine assays

The amounts of secreted interleukins IL-6, IL-8, and MCP-1 in the collected supernatant of intestinal epithelial cell samples were determined by ELISA. Commercially available ELISA kits (Diaclone) for the quantitation of cytokines were used as described in the manufacturer’s instructions. The absorbance was measured at 450 nm using a BioTek Synergy HT Multi-Mode microplate reader (BioTek Instruments Inc., Vermont, USA). All the quantifications were performed in triplicate. Such as in the absence of bacteria, intestinal cells release small amounts of IL-6, IL-8, and MCP-1 (Bahrami et al., 2011; Candela et al., 2008; Ferrero, Fossati, Rumbo, & Baldi, 2012), titers of cytokine released by Caco-2/HT-29 cells (pg/mL) were determined experimentally.

2.7. Characterization of the phenolic composition of GSE

Individual phenolic compounds on GSE extract were determined by HPLC analyses and mass spectrometry (MS) detection. All HPLC analyses were carried out on a Hewlett Packard Agilent 1200 Series liquid chromatography system equipped with a quaternary pump and a photodiode array detector (DAD) (Agilent Technologies, Waldrom, Germany). The

| Procedure                          | Strain | Cell line | GSE (mg/mL) | Pre-treatment time | Infection time |
|-----------------------------------|--------|-----------|-------------|-------------------|---------------|
| Cytokine stimulation              | LP1    | HT-29/Caco-2 | --          | --                | 3, 6, 24 h    |
| Strain and cell line selection    | LP1    | HT-29/Caco-2 | --          | --                | 24 h          |
|                                  | 118    | HT-29/Caco-2 | --          | --                | 24 h          |
|                                  | CN1    | HT-29/Caco-2 | --          | --                | 24 h          |
|                                  | CN2    | HT-29/Caco-2 | --          | --                | 24 h          |
|                                  | CIAL1  | HT-29/Caco-2 | --          | --                | 24 h          |
| Co-treatment with GSE            | LP1    | HT-29    | 0.5, 0.2, 0.06, 0.02 | --              | 24 h          |
| Pre-treatment with GSE           | LP1    | HT-29    | 0.5, 0.2, 0.06, 0.02 | 3 h             | 24 h          |
column used was a Phenomenex Luna C18 column (4.6 × 150 mm, 5 μm) (Phenomenex, California, USA) which was set thermostatically at 25°C. Chromatographic data were acquired and processed using an Agilent Chemstation for LC 3D system (Rev. B.04.01) (Agilent Technologies). The HPLC method conditions were as described by Silvan et al. (2013). Briefly, the binary mobile phase used for analyses were aqueous 4.5% formic acid (A) and HPLC-grade acetonitrile (B) at a flow rate of 0.5 mL/min. The elution was starting with 10% B, the gradient was 20% B from 0 to 20 min, 25% B from 20 to 30 min, and 35% B from 30 to 50 min. Detection wavelengths were 280, 320, 440, and 520 nm and samples were analysed in triplicate. Peaks were identified by comparing their retention time and UV-vis spectra with the reference compounds, and the data were quantified using the corresponding curves of the reference compounds as standards. In order to confirm the identity of the recorded compounds, additional analyses were performed by using HPLC-MS. For MS analysis an Agilent 1100 series liquid chromatograph/mass-selective detector equipped with a quadrupole (G1946D, Agilent Technologies) mass spectrometer was used, employing the same conditions described above for separation and elution. Electrospray ionization in the positive mode was used. The electrospray capillary voltage was set to 2500 V, with a nebulizing gas flow rate of 12 L/min and a drying gas temperature of 150°C.

2.8. Statistical analysis

The results were reported as means ± standard deviations (SD) performed in triplicate. The data were processed by analysis of variance (ANOVA) with a post hoc Duncan test. Differences were considered significant at \( p < .05 \). All statistical tests were performed with IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

3.1. Induction of pro-inflammatory cytokines in human intestinal epithelial cells by campylobacter

Previous studies have shown that *C. jejuni* increase the expression of a number of cytokines with chemoattractant and pro-inflammatory functions in human intestinal epithelial cells (Bahrami et al., 2011; Borrmann et al., 2007; Stašová et al., 2015). We investigated the production of pro-inflammatory cytokines (IL-6, IL-8, and MCP-1) released from human intestinal epithelial cells (Caco-2 and HT-29) in response to *C. jejuni* stimuli. HT-29 and Caco-2 monolayers cells represent a well characterized model to study the enterocyte immune response to bacterial infection (Bahrami et al., 2011; Németh, Halász, Baráth, & Gálfi, 2007). Cells were incubated with *C. jejuni* for 3, 6, and 24 h, and the levels of the cytokines production in the culture supernatants were then measured by ELISA. Figure 1 shows the production levels of cytokines by intestinal cells after *C. jejuni* infection. In both cell lines *C. jejuni* markedly induced secretion of IL-6, IL-8, and MCP-1. Induction levels for IL-8 and MCP-1 were higher than IL-6 in both intestinal cell lines. The higher induction of IL-8 and MCP-1 production by Caco-2 and HT-29 cells after infection with *C. jejuni* was in accordance with previous report of bacteria-induced production of cytokines by human intestinal epithelial cells (Bermudez-Brito et al., 2012; Saarinen et al., 2002; Saegusa, Totsuka, Kaminogawa, & Hosoi, 2007). While IL-8 effectively attracts neutrophils,
MCP-1 is a potent chemoattractant for monocytes and T lymphocytes, and activates basophils. Thus, they play an important role in initiating the first line defense against pathogenic bacteria in the intestinal mucosa. At 3 h and 6 h post-infection with *Campylobacter*, the

Figure 1. Pro-inflammatory cytokine IL-6 (A), IL-8 (B), and MCP-1 (C) secretion by human intestinal epithelial cell lines (Caco-2 and HT-29) induced by *C. jejuni* LP1 after 3, 6, and 24 h of exposure. Values are the mean ± SD (n = 3). Asterisks indicate statistical difference (p < .05) compared with non-infected cells for each experimental time (*Caco-2 and **HT-29).
levels of the pro-inflammatory markers were sequentially increased, reaching the maximum values at 24 h. Thus, the secretion of cytokines after \textit{C. jejuni} stimulation was time dependent. Cytokine production was higher in HT-29 than in Caco-2 cells, showing different levels of secretion depending of the epithelial cells. This is consequent with the cells origin. Caco-2 cells have evolved to tolerate antigenic challenge and maintain gut homeostasis, while HT-29 are not colonized in healthy mucosae and do not normally encounter live bacteria, and should therefore be potentially more susceptible to antigenic challenge (Bahrami et al., 2011). Other authors have previously described that \textit{Campylobacter} can elicit cytokine production from intestinal epithelial cells and other cell types (Bostanci et al., 2007; Hu & Kopecko, 2008), being the degree of this response related with several variables, among them the strain used. Therefore, we evaluate the levels of IL-6, IL-8, and MCP-1 in infected cell cultures of Caco-2 and HT-29 with six different \textit{Campylobacter} strains. The results obtained are shown in Figure 2. Strains isolated from clinical samples (LP1, 118, and CIII) produced significant higher levels of cytokines than the food-chain strains (CN1, CN2, and CIAL1) in both cell lines, being LP1 the strain with greater levels for the three tested cytokines in HT-29 cells. These results are in accord with previous studies which demonstrate the variability in the levels of IL-8 secretion in human embryo intestinal epithelial cells after exposure to different clinical isolates of \textit{C. jejuni} (Hickey, Baqar, Bourgeois, Ewing, & Guerry, 1999). In this study, the authors found that the highest invasive strains were also those which induce the highest levels of IL-8 release. Although new research are needed using more strains, the results obtained in this work suggest that infective isolates coming from campylobacteriosis patients could be associated with the production of higher levels of some pro-inflammatory cytokines in Caco-2 and HT-29 epithelial cells. Differential expression of cytokines with each strain could be attributed to the organisms employing different virulence factors or strategies to facilitate the infection (Bahrami et al., 2011). As same as above, cytokine levels were higher in HT-29 than in Caco-2 cells in all cases. Accordingly, HT-29 cells and \textit{C. jejuni} LP1 strain were selected to study the effect of GSE in the epithelial cell immune response against \textit{Campylobacter}, setting the experimental time for cytokine induction in 24 h.

### 3.2. Effect of GSE on the induction of pro-inflammatory cytokines in human intestinal epithelial cells by campylobacter

The effect of GSE on the induction of pro-inflammatory cytokines in HT-29 epithelial cells in co-treatment experiments (GSE and \textit{C. jejuni} added at the same time) is showed in Figure 3. The addition of GSE (GSE control) to the cells did not induce pro-inflammatory effects (Figures 3 and 4). Most of the concentrations of GSE tested (from 0.02 mg/mL to 0.5 mg/mL) were highly effective, reducing significantly the cytokine secretion of IL-6, IL-8, and MCP-1 above 90% respect to control (GSE 0 mg/mL), except for IL-8 at GSE concentration of 0.02 mg/mL, with a reduction of 36.2%. In the pre-treatment experiments, where HT-29 cells were previously incubated 3 h with GSE and then infected with \textit{Campylobacter} for 24 h, the behavior was similar, although the extract efficacy decreased as far as GSE concentration did (Figure 4). The results obtained showed that the anti-inflammatory effect of GSE significantly remains at concentrations between 0.06 mg/mL and 0.5 mg/mL, while the activity is lost at the low concentration tested (0.02 mg/mL). This result suggests that a portion of the anti-inflammatory molecules of GSE are modified during the pre-treatment, decreasing its activity. Anyway, the activity of GSE as
Figure 2. Pro-inflammatory cytokine IL-6 (A), IL-8 (B), and MCP-1 (C) secretion by human intestinal epithelial cell lines (Caco-2 and HT-29) induced by *C. jejuni* strains after 24 h of exposure. Values are the mean ± SD (*n* = 3). Asterisks indicate statistical difference (*p* < .05) compared with its cellular control (*Caco-2* and **HT-29).
Figure 3. Pro-inflammatory cytokine IL-6 (A), IL-8 (B), and MCP-1 (C) secretion by human intestinal epithelial HT-29 cell line induced by the co-treatment of GSE and C. jejuni infection for 24 h. Values are the mean ± SD (n = 3). Asterisk indicate statistical difference (p < .05) compared with the sample without GSE (0 mg/mL).
Figure 4. Pro-inflammatory cytokine IL-6 (A), IL-8 (B), and MCP-1 (C) secretion by human intestinal epithelial HT-29 cell line induced by pre-treatment with GSE for 3 h and C. jejuni infection for 24 h. Values are the mean ± SD (n = 3). Asterisk indicate statistical difference (p < .05) compared with the sample without GSE (0 mg/mL).
inflammation modulator against *Campylobacter* infection could be considered as relevant, considering that the effective concentration of GSE is below 0.1 mg/mL in most of the cases, and this range is very promising from the practical point of view (Rios & Recio, 2005). Several bioactive effects have been reported for GSE in addition to the antioxidant and antimicrobial properties. Among them, there are some evidences about its anti-inflammatory capacities. For example, Wang et al. (2013) demonstrated that dietary GSE supplementation exerted protective effects in different indices of the inflammatory bowel disease (IBD) in IL-10 deficient mice, through several mechanisms including modulating gut microflora and blocking gut inflammatory response. Also, GSE diet supplementation was able to restore the elevated C-reactive protein (CRP) levels in the obese mice to a normal range found in the lean mice, showing and improvement in the inflammatory immune response due to GSE consume (Hogan et al., 2011). In the other hand, GSE significantly decreased the TNF-α-induced inflammatory status of human umbilical vein endothelial cells (HUVEC), suggesting that consumption of GSE could be beneficial to inflammatory atherosclerosis (Chao et al., 2011). More recently, it has been shown that grape-derived products, such as GSE, have a relevant anti-inflammatory effect in the pig intestine even under *in vivo* conditions (Fiesel, Gessner, Most, & Eder, 2014; Gessner et al., 2013). In spite of these examples and other evidences related with the anti-inflammatory role of GSE (Derry et al., 2013; Gessner et al., 2012; Li et al., 2014; Yang et al., 2014), from our knowledge this is the first report showing its effectiveness against inflammatory response in *Campylobacter* infection.

### 3.3. Determination of individual phenolic compounds in GSE

In Table 3 are shown the main individual phenolic compounds identified in the GSE. The main group of phenolic compounds consisted of catechins and proanthocyanidins,

| Compound                  | mg/L     |
|---------------------------|----------|
| **Flavonols**             |          |
| Quercetin-3-glucoside     | 71.7 ± 0.5 |
| Myricetin-3-glucoside     | 22.5 ± 1.4 |
| Kaempferol-3-glucoside    | 16.4 ± 1.2 |
| **Phenolic acids**        |          |
| Gallic acid               | 29.1 ± 0.7 |
| Protocatechuic acid       | 36.1 ± 1.3 |
| Caftaric acid             | 299.8 ± 4.8 |
| Homovanillic acid         | 2.9 ± 0.1 |
| Clorogenic acid           | 35.5 ± 0.1 |
| **Catechins and proanthocyanidins** |    |
| Catequin (Cat)            | 274.1 ± 3.1 |
| Epicatechin (Ec)          | 381.5 ± 5.6 |
| B1 (Cat-Ec)               | 167.4 ± 3.6 |
| B2 (Ec-Ec)                | 486.2 ± 4.7 |
| Ec-Ec-Cat                 | 475.5 ± 5.3 |
| Epicatechin gallate       | 6.4 ± 0.6 |
| **Anthocyanins**          |          |
| Delphinidin-3-glucoside   | 2.8 ± 0.2 |
| Peonidin-3-glucoside      | 9.1 ± 0.1 |
| Malvidin-3-glucoside      | 0.2 ± 0.0 |

Note: Values are the means ± SD (*n* = 3).
representing around the 80% of the phenolic compounds. Phenolic acids, flavonols, and anthocyanins were the rest of phenolic families identified. This composition is similar to the most of GSE reported in previous works (Mingo, Silván, & Martínez-Rodriguez, 2016; Silvan et al., 2013). Such as catechins and their polymers are the main bioactive molecules associated to GSE composition, they could be significantly involved in the anti-inflammatory activity. Others have been previously demonstrated that cocoa and green tea procyanidins can have an active role attenuating inflammation (Albuquerque, Marínovic, Morandi, Bolin, & Otton, 2016; Bitzer et al., 2015). Compounds such as epicatechin and procyanidins B1 and B2 can also suppress the production of pro-inflammatory cytokines (Al-Hanbali et al., 2009; Jung, Triebel, Anke, Richling, & Erkel, 2009). This fact is also consistent with the loss of some anti-inflammatory activity in the experiments with pre-incubation, because epithelial cells can transform polyphenols through several mechanisms (Kamiloglu, Capanoglu, Grootaert, & Van Camp, 2015).

Summarizing, the food-grade category of GSE, together with its relevant antimicrobial properties against Campylobacter (Silvan et al., 2013) and the anti-inflammatory properties demonstrated in this work, makes this natural product a putative nutritional tool for use in the treatment of campylobacteriosis, which could contribute to improve disease prevention or reduce disease severity.

4. Conclusions

The present data update our knowledge about intestinal cells response after C. jejuni exposure. We detected that C. jejuni also induces an important pro-inflammatory response in intestinal cells; involving IL-6, IL-8, and MCP-1. The results also describe how GSE interact with C. jejuni and reduce pro-inflammatory cytokines secretion in human intestinal epithelial cells HT-29 in a dose-dependent manner. These data contribute towards our understanding of how GSE can be used to modulate the pro-inflammatory response in inflammatory C. jejuni infections.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Al-Hanbali, M., Ali, D., Bustami, M., Abdel-Malek, S., Al-Hanbali, R., Alhussainy, T., … Matalka, K. Z. (2009). Epicatechin suppresses IL-6, IL-8 and enhances IL-10 production with NF-κB nuclear translocation in whole blood stimulated system. *Neuroendocrinology Letters*, 30, 131–138.

Albuquerque, K. F. F. S., Marinovic, M. P., Morandi, A. C., Bolin, A. P., & Otton, R. (2016). Green tea polyphenol extract in vivo attenuates inflammatory features of neutrophils from obese rats. *European Journal of Nutrition*, 55, 1261–1274.

Allos, B. M. (2001). *Campylobacter jejuni* infections: Update on emerging issues and trends. *Clinical Infectious Diseases*, 32, 1201–1206.

Badet, C. (2011). Nuts & seeds in health and disease prevention. In V. R. Preedy, R. R. Watson, & V. B. Patel (Eds.), *Antibacterial activity of grape (Vitis vinifera, Vitis rotundifolia) seeds* (pp. 545–552). London: Academic Press.

Bahrami, B., Macfarlane, S., & Macfarlane, G. T. (2011). Induction of cytokine formation by human intestinal bacteria in gut epithelial cell lines. *Journal of Applied Microbiology*, 110, 353–363.

Bermudez-Brito, M., Muñoz-Quezada, S., Gomez-Llorente, C., Matencio, E., Bernal, M. J., Romero, F., & Gil, A. (2012). Human intestinal dendritic cells decrease cytokine release against *Salmonella* infection in the presence of *Lactobacillus paracasei* upon TLR activation. *PLoS One*, 7, e43197.

Bibi, S., Kang, Y., Yang, G., & Zhu, M. J. (2016). Grape seed extract improves small intestinal health through suppressing inflammation and regulating alkaline phosphatase in IL-10-deficient mice. *Journal of Functional Foods*, 20, 245–252.

Bitzer, Z. T., Gilsan, S. L., Dorenkott, M. R., Goodrich, K. M., Ye, L., O’Keefe, S. F., … Neilson, A. P. (2015). Cocoa procyanidins with different degrees of polymerization possess distinct activities in models of colonic inflammation. *The Journal of Nutritional Biochemistry*, 26, 827–831.

Borrmann, E., Berndt, A., Hänel, I., & Köhler, H. (2007). *Campylobacter*-induced interleukin-8 responses in human intestinal epithelial cells and primary intestinal chick cells. *Veterinary Microbiology*, 124, 115–124.

Bostanci, N., Allaker, R. P., Belibasakis, G. N., Rangarajan, M., Curtis, M. A., Hughes, F. J., & McKay, I. J. (2007). Porphyromonas gingivalis antagonises *Campylobacter rectus* induced cytokine production by human monocytes. *Cytokine*, 39, 147–156.

Candela, M., Perna, F., Carnevali, P., Vitali, B., Ciati, R., Gionchetti, P., … Brigidi, P. (2008). Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production. *International Journal of Food Microbiology*, 125, 286–292.
Li, Y. P., Vegge, C. S., Brøndsted, L., Madsen, M., Ingmer, H., & Bang, D. D. (2011). Campylobacter jejuni induces an anti-inflammatory response in human intestinal epithelial cells through activation of phosphatidylinositol 3-kinase/Akt pathway. *Veterinary Microbiology*, 148, 75–83.

Li, X., Yang, Y., Liu, S., Yang, J., Chen, C., & Sun, Z. (2014). Grape seed extract supplementation attenuates the heat stress-induced responses of jejunum epithelial cells in Simmental × QinChuan steers. *British Journal of Nutrition*, 112, 347–357.

MacCallum, A. J., Harris, D., Haddock, G., & Everest, P. H. (2006). Campylobacter jejuni-infected human epithelial cell lines vary in their ability to secrete interleukin-8 compared to in vitro-infected primary human intestinal tissue. *Microbiology*, 152, 3661–3665.

Mandic, A., Dilas, S. M., Cetkovic, G. S., Canadianovic-Brunet, J. M., & Tumbas, V. T. (2008). Polyphenolic composition and antioxidant activities of grape seed extract. *International Journal of Food Properties*, 11, 713–726.

Mingo, E., Silván, J. M., & Martinez-Rodriguez, A. J. (2016). Selective antibacterial effect on Campylobacter of a winemaking waste extract (WWE) as a source of active phenolic compounds. *LWT – Food Science and Technology*, 68, 418–424.

Németh, E., Halász, A., Baráth, A., & Gálfi, P. (2007). Influence of lactic acid bacteria and their spent culture supernatant on hydrogen peroxide-induced interleukin-8 synthesis and necrosis of Caco-2 cells. *Food and Agricultural Immunology*, 18, 95–105.

Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100, 80–84.

Saarinen, M., Ekman, P., Ikeda, M., Virtala, M., Grönberg, D. T., Yu, Y., … Granfors, K. (2002). Invasion of Salmonella into human intestinal epithelial cells is modulated by HLA-B27. *Rheumatology*, 41, 651–657.

Saegusa, S., Totsuka, M., Kaminogawa, S., & Hosoi, T. (2007). Cytokine responses of intestinal epithelial-like Caco-2 cells to non-pathogenic and opportunistic pathogenic yeasts in the presence of butyric acid. *Bioscience, Biotechnology, and Biochemistry*, 71, 2428–2434.

Silvan, J. M., Mingo, E., Hidalgo, M., de Pascual-Teresa, S., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2013). Antibacterial activity of a grape seed extract and its fractions against Campylobacter spp. *Food Control*, 29, 25–31.

Stadnyk, A. (2002). Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. *Canadian Journal of Gastroenterology*, 16, 241–246.

Stašová, D., Husáková, E., Bobíková, K., Karaffová, V., Levkutová, M., & Levkut, M. (2015). Expression of cytokines in chicken peripheral blood mononuclear cells after stimulation by probiotic bacteria and Campylobacter jejuni in vitro. *Food and Agricultural Immunology*, 26, 813–820.

Wang, H., Xue, Y., Zhang, H., Huang, Y., Yang, G., Du, M., & Zhu, M. J. (2013). Dietary grape seed extract ameliorates symptoms of inflammatory bowel disease in IL10-deficient mice. *Molecular Nutrition & Food Research*, 57, 2253–2257.

Yang, G., Wang, H., Kanga, Y., & Zhu, M. J. (2014). Grape seed extract improves epithelial structure and suppresses inflammation in ileum of IL-10-deficient mice. *Food & Function*, 5, 2558–2563.