

Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro

Laura Soler-Vasco, Rafael Hermes, Théo A. Niewold

▶ **To cite this version:**

Laura Soler-Vasco, Rafael Hermes, Théo A. Niewold. Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 2016, 07 (11), pp.1531-1537. 10.4236/ajps.2016.711144 . hal-01605747

HAL Id: hal-01605747
https://hal.science/hal-01605747

Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

American Journal of Plant Sciences, 2016, 7, 1531-1537
Published Online August 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.711144

Scientific Research Publishing

Macleaya cordata Extract Reduces Inflammatory Responses of Intestinal Epithelial Cells *in Vitro*

Laura Soler[1,2], Rafael Hermes[3,4], Theo A. Niewold[1*]

[1]Nutrition and Health Unit, Department of Biosystems, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Leuven, Belgium
[2]INRA Val de Loire, Nouzilly, France
[3]Grup de Nutrició, Maneigi Benestar, Department de Ciència Animal idels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Spain
[4]DSM Nutritional Products AG, Kaiseraugst, Switzerland
Email: *theo.niewold@biw.kuleuven.be

Received 2 June 2016; accepted 31 July 2016; published 3 August 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

***Background*: The EU ban on antimicrobial growth promoters (AGP) has initiated a search for non-antibiotic alternatives. It has been demonstrated that certain antibiotics and non-antibiotic alternatives enhance growth by inhibiting inflammatory cells, *i.e.* neutrophils and macrophages in the intestine. There is very little information on the effect of anti-inflammatory compounds on intestinal epithelial cells, which are known to play an important role in intestinal inflammatory responses. In order to establish this, a porcine intestinal epithelial cell line (IPEC J2) was incubated with an adherent enterotoxigenic *E. coli* (ETEC) to stimulate inflammation, using a non-pathogenic non-adherent *E. coli* (EC) as a control. The influence of the presence of the anti-inflammatory compounds *Macleaya cordata* extract (MCE) and acetylsalicylic acid (ASA) on inflammatory transcriptional responses was studied. *Results*: ETEC induced a strong inflammatory response as was most evident from the expression of IL-1β, IL-8 and TNF-α, whereas EC induced IL-1β only. Co-incubation with MCE and ASA significantly reduced the responses of the pro-inflammatory cytokines, similarly for IL-1β and TNF-α, but ASA was more effective than MCE in reducing the IL8 response. *Conclusions*: The present results suggest that the *in vivo* anti-inflammatory growth promoting effects of AGP and effective alternatives to AGP such as MCE and ASA are not restricted to inflammatory cells and also involve the more abundant enterocytes. This suggests a major role for epithelial cells in growth promotion livestock, and it further supports the notion that effective alternatives to AGP should have anti-inflammatory activity.**

*Corresponding author.

How to cite this paper: Soler, L., Hermes, R. and Niewold, T.A. (2016) *Macleaya cordata* Extract Reduces Inflammatory Responses of Intestinal Epithelial Cells *in Vitro*. *American Journal of Plant Sciences*, **7**, 1531-1537.
http://dx.doi.org/10.4236/ajps.2016.711144

Version postprint

Comment citer ce document :
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

Keywords

Enterocyte, IPEC-J2, Cell Culture, Inflammation, Growth Promotion

1. Introduction

Antimicrobial growth promoters (AGP) are antibiotics added to feed in sub-therapeutic concentrations. In 2006 they were banned in the European Union, which has led to a search for effective alternatives. This search was hampered by the lack of a plausible mechanism for AGP. Initially, the growth promoting effect of AGP in livestock was attributed to their antibiotic character [1]. This is unlikely, mainly because of the fact that AGP are antibiotics in sub-therapeutic concentrations. In 2007, it was proposed that AGP rather work by direct inhibition of inflammatory responses, thus saving energy for growth [2]. It also explained why AGP still worked in the presence of wide spread antibiotic resistance [3]. Further support for the concept of direct inhibition of inflammation by AGP came from recent studies in pigs and poultry [4]-[7]. In the original publication on the anti-inflammatory concept the major target cells were thought to be intestinal macrophages and neutrophils because there was ample *in vitro* and *in vivo* evidence on inactivation of the latter by AGP antibiotics [2]. However, it is clear that the major cell types in the intestines are enterocytes which play an important role in modulating intestinal inflammatory responses working in close concert with inflammatory cells (for recent reviews see [8] [9]). Recently, we have demonstrated that growth promoting effects of AGP and the phytobiotic alternative *Macleaya cordata* extract (MCE) coincide with a reduction of inflammatory responses in small intestinal mucosa in broilers [10]. Because the mucosa contains a multitude of different cell types, no conclusions could be drawn on the role of enterocytes. Therefore, in the current study we tried to establish the inflammatory response of a monoculture of enterocytes to MCE using the non-antibiotic anti-inflammatory compound acetylsalicylic acid (ASA) [11] with known growth promoting activity [12] as a control. MCE is an extract of *Macleaya cordata* (commonly known as plume poppy), and the active anti-inflammatory component is sanguinarine [10]. Co-culture with enterotoxigenic *E. coli* (ETEC) was used to stimulate inflammation and gene expression was compared with co-culture with a non-pathogenic non-adhesive *E. coli* strain (EC), using the porcine intestinal cell line IPEC-J2.

2. Material and Methods

2.1. Bacterial Strains

Two strains of *E. coli* were used in this study. An enterotoxigenic F4 fimbriae type *E. coli* K88ac (MUN 287), (ETEC; [13]) was used to stimulate inflammation, whereas a non-fimbriated *E. coli* strain (G58-1), (EC; [13]) was used as control. Both ETEC and EC were kindly donated by the Professor Rodney A. Moxley (University of Nebraska, Lincoln) and were grown in Luria Bertani Broth at 37˚C for 18 h before use under low agitation to reach the concentration of 1 to 2 × 10^8 CFU/mL. For each experiment, the bacterial solution was adjusted to 2 × 10^8 CFU/mL using the optic an density at 650 nm, and samples were plated on Luria agar to confirm the number of bacteria.

2.2. Cell Culture

The IPEC-J2 (epithelial cell line derived from the jejunum of neonatal piglets), were kindly donated by the Professor Anthony Blikslager (North Carolina State University). Cells were maintained following previously established protocols, and for the gene expression assay, cells were seeded into 6-well plates in a 2-mL volume/well, containing 2 - 4 × 10^5 cells each. Cells were used at confluence after 3 to 4 d [14]. Experiments were performed in triplicate. Anti-inflammatory treatments included a commercial *Macleaya cordata* extract (MCE) (Sangrovit WS SV Phytobiotics GmbH, Eltville, Germany containing 12,235 mg/kg of sanguinarine and 4073 mg/kg of chelerythrine, of which sanguinarine is the active component [10]) and acetylsalicylic acid (ASA, Sigma Aldrich, St. Louis, USA), both diluted in sterile PBS at 0.001% (w/v), which is in the range of the intestinal concentration expected by in feed use of the compounds as AGP. One mL of each *E. coli* strains cultures (2 × 10^8 CFU/mL) was mixed with 1 mL of MCE, ASA or PBS (control) solutions and pre-incubated for 30 minutes at RT [14]. After this step, the solutions were added to confluent monolayers of IPEC-J2 and incubated for 2 h at

Version postprint

Comment citer ce document :
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

37˚C with 5% of CO_2. Then, cells from all treatments were washed twice with sterile PBS and used for RNA extraction [14]. Cells were incubated for no more than 2 h to allow for sufficient gene induction whilst preventing artefacts caused by cell death as a result of bacterial overgrowth.

2.3. RNA Isolation and Quantitative PCR Analysis

RNA extraction was performed by adding 1 mL of TriPure Isolation Reagent (Roche Applied Science) to each well and following the manufacturer's instructions for centrifuge-based extraction. A Turbo DNA-free kit (Applied Biosystems) was used to digest contaminating DNA. The integrity of the RNA was checked by analyzing 0.5 µg on a 1% (w/v) agarose gel. RNA concentration and purity were determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity was assessed using a Bioanalyser 2100 (Agilent). cDNA was produced then by using a iScript cDNA Synthesis Kit (Bio-Rad) following the manufacturer's instructions. Real-time quantitative PCR were used to quantify the gene products of interest (TLR-4, TLR-5, IL-1*β*, IL-8, IL-10; and TNF-*α*) relative to the quantity of Cyclophilin A mRNA in total RNA isolated from cultured IPEC-J2. Cyclophilin A was used as a reliable housekeeping gene for low abundant transcripts in expression studies in different pig tissues using SYBR green quantitative PCR [15]. PCR reactions were carried out in triplicate in 96-well plates using the iQ5 Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Each reaction included the appropriate forward and reverse primers (200 nM) (**Table 1**), 5 µL of cDNA (not included in non template controls) and 12.5 µL of iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) The following thermal protocol was performed: 30 cycles at 94˚C for 30 s, 57˚C for 30 s and 72˚C for 45 s. A standard curve including TLRs, cytokine and housekeeping genes was generated using serial dilutions of a pooled RNA. Relative abundance of TLRs, cytokines and chemokines gene expression was determined from real-time PCR data of IPEC-J2 cells by the ratio (R) equation [15]. The ratios were normalized using the results of a negative control treatment (cell line without the bacterial challenge).

2.4. Statistical Analysis

The statistical software program Graph Pad Prism 5 for Windows (GraphPad software) was used to carry out all statistics. Data followed a normal distribution as assessed by the D'Agostino-Pearson normality test. Differences in gene expression between ETEC and EC treated cells with MCE, ASA and PBS were compared with each other by one-way ANOVA and Tukey's multiple comparison post-tests. The significance level was set at $P < 0.05$.

3. Results

The results are given in **Figure 1**. ETEC compared to EC induced higher mRNA levels of TLR-4 and TLR-5,

Table 1. Primers for real-time polymerase chain reaction.

Gene name	Accession number	Sequence (5' - 3')	Efficiency	R^2	Reference
Cyclophilin-A-for	JX523419	CCTGAACATACGGGTCCTG	2.00	0.98	[15]
Cyclophilin-A-rev		AACTGGGAACCGTTTGTGTTG			
TLR4-for	AB188301	GCCATCGCTGCTAACATCATC	1.98	0.99	[15]
TLR4-rev		CTCATACTCAAAGATACACCATCGG			
TLR5-for	NM_001123202	CAGCGACCAAAACAGATTGA	2.00	0.99	[20]
TLR5-rev		TGCTCACCAGACAGACAACC			
IL1*β*-for	NM_214055	GGCCGCCAAGATATAACTGA	1.99	0.98	[15]
IL1*β*-rev		GGACCTCTGGGTATGGCTTTC			
IL8-for	M86923	TTCGATGCCAGTGCATAAATA	2.02	0.97	[15]
IL8-rev		CTGTACAACCTTCTGCACCCA			
IL10-for	L20001	CAGATGGGCGACTTGTTG	2.00	0.99	[20]
IL10-rev		ACAGGGCAGAAATTGATGAC			
TNF*α*-for	NM_214022	CGCCCACGTTGTAGCCAATGT	2.00	0.99	[15]
TNF*α*-rev		CAGATAGTCGGGCAGGTTGATCTC			

Version postprint

Comment citer ce docum
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

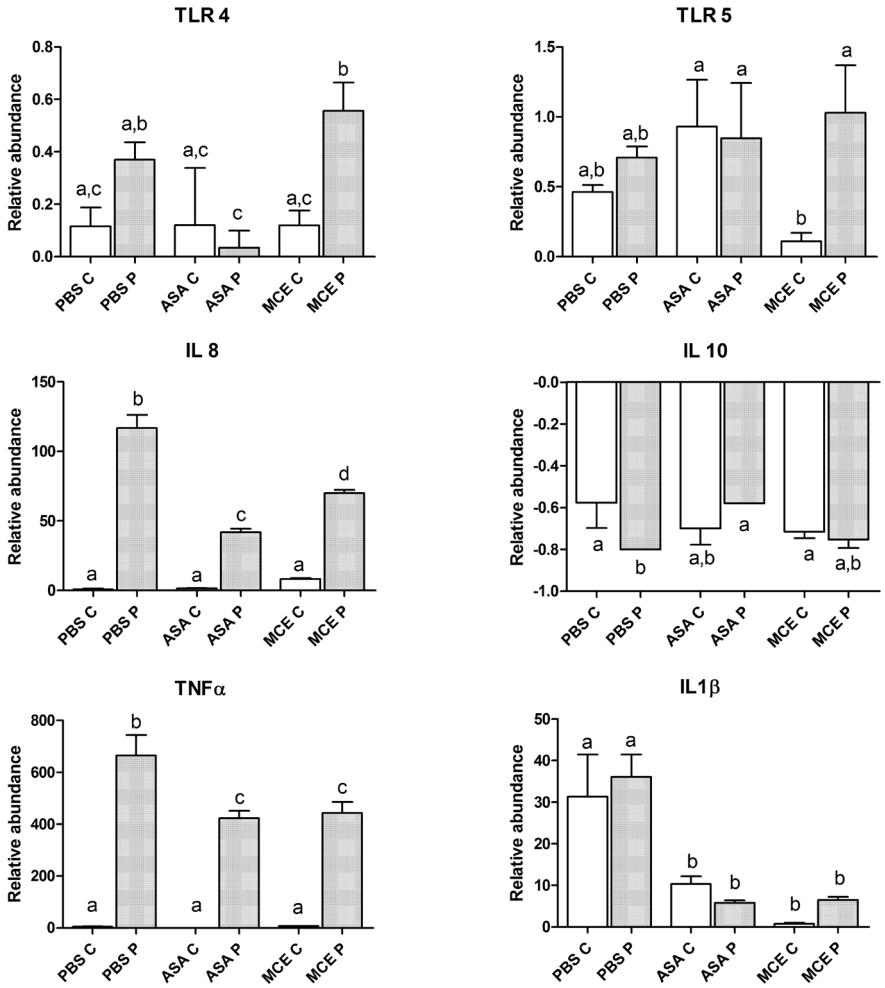


Figure 1. The response of IPEC-J2 cells to challenge with two strains of *E. coli* in the presence or absence of anti-inflammatory compounds. C is the non-pathogenic *E. coli* control, P the pathogenic *E. coli* (ETEC). ASA is acetylsalicylic acid, MCE is *Macleaya cordata* extract. Experiments were performed in triplicate (n = 3). Errors bars represent the standard deviation of the mean. Letters indicate the result of statistical testing. Treatments not sharing letters are significantly different ($P < 0.05$).

although differences were not significant. However, lower levels of IL-10 ($P < 0.01$), and higher levels of IL-8 ($P < 0.001$), and TNF-α ($P < 0.001$) mRNA were found in ETEC-challenged cells compared to EC challenged cells, whereas levels of IL-1β were not different. In ASA-treated cells, no differences in expression of TLR-4, TLR-5, IL-10 and IL-1β were found in ETEC-challenged cells compared to EC, but the first showed higher mRNA levels of IL-8 ($P < 0.001$) and TNF-α ($P < 0.001$). In ETEC-challenged cells treated with MCE, higher mRNA levels were found compared to control (EC) cells for TLR-4 ($P < 0.001$), TLR-5 ($P < 0.001$), IL-8 ($P < 0.001$), and TNF-α ($P < 0.001$), while IL-10 and IL-1β expression was not different. Compared to PBS-treated cells, ASA caused a reduction in TLR-4 ($P < 0.001$), IL-8 ($P < 0.001$), IL-10 ($P < 0.01$), TNF-α ($P < 0.001$) and IL-1β ($P < 0.001$) mRNA levels in ETEC-challenged cells. A lower expression of IL-8 ($P < 0.001$), TNF-α ($P < 0.001$) and IL-1β ($P < 0.001$) was detected in ETEC-challenged cells treated with MCE compared with PBS. Differences in pro-inflammatory gene expression between the two anti-inflammatory treatments in ETEC-challenged cells were evident for TLR-4 and IL-8, which showed lower expression in ASA-treated cells than in cells exposed to MCE ($P < 0.001$).

4. Discussion

The IPEC-J2 cell line (intestinal porcine epithelial cells), isolated from jejunum of neonatal piglets, is a reliable

Version postprint
Comment citer ce docum
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

model for the *in vitro* study of the swine small intestine [16]. It is also very useful for studying the inflammatory responses to F4 fimbriated ETEC mainly because it expresses the F4-specific receptor [17] [18]. After challenge with ETEC F4, IPEC-J2 cells express the Toll-like receptor-4 (TLR-4), as well as TLR-5, leading to up regulation of IL-1β, IL-8 and TNFα, and down regulation of IL-10 [14]. ETEC strains without F4 fimbriae lead to lower expression of interleukins, and non-pathogenic, non-F4 fimbriated EC induce the lowest responses in particular concerning IL-8 [14] [18].

Concerning the non-treated controls, results were largely similar to those of the earlier study [14]. For TLR-4 and TLR-5 no large differences were found between the bacterial strains, and the effect of the anti-inflammatory compounds on the expression of these genes is limited too. IL-10 was down regulated in response to ETEC, but no effect of ASA and MCE was seen. No difference was found between the EC strains for IL-1β, however, a clear significant down regulation of IL-1β expression was seen in the presence of ASA and MCE. A striking difference between the bacterial strains was seen for IL-8 and TNF-α as before [14]. This is consistent with the strong effect of F4 fimbriated ETEC on IL8 expression of IPEC-J2 [18]. Furthermore, as was the case for IL-1β, a clear significant reduction of IL-8 and TNF-α expression by ASA and MCE of ETEC compared to the untreated controls was found. It should be realized that cytokine mRNA responses as quantified here by PCR do not always translate into equal amounts of active secreted cytokine protein, and results should be interpreted with caution. However, it is deemed unlikely that the strong and consistent response of the three different pro-inflammatory cytokines to ASA and MCE were not functionally relevant.

The mechanism by which ASA exerts its activity in cells is not entirely clear [11], and the same is the case for MCE [10]. Still, MCE and ASA have clear anti-inflammatory effects on different cell types including enterocytes as demonstrated here. This is consistent with the anti-inflammatory effects described by others *in vitro* and *in vivo*, although there are certainly differences concerning the expression of particular anti-inflammatory genes. For instance, as opposed to *in vitro*, in jejunal mucosa of broilers there was no effect of in feed MCE on IL-10 expression, and only a small quadratic effect on IL-1β [10]. Differences with other *in vitro* studies may be due to different cell types, incubation conditions and time *et cetera*. Similarly, large differences with *in vivo* conditions are apparent. It can also be argued that species differences may exist between pig and chicken. However, it has been demonstrated that chicken immunological responses in the small intestine are largely similar to mammals (e.g. [19]). Furthermore, a recent study in piglets showed that MCE promoted growth while reducing the plasma levels of acute phase proteins [20], indicating a similar anti-inflammatory mechanism as in chicken.

Thus it appears that MCE (and other growth promoters) inhibit inflammation *in vitro* as well as *in vivo* [10] [21]. Inflammation is known to reduce growth (through inappetance and muscle catabolism), which explains why effective growth promoters must be inhibitors of inflammatory responses, thus sparing energy for growth [2].

5. Conclusion

The present *in vitro* results indicate that the anti-inflammatory effect of growth promoters *in vivo* is not likely restricted to inflammatory cells such as macrophages, and also affects the more abundant enterocytes. Thus epithelial cells may play an important role in the growth promoting effects of anti-inflammatory compounds. It is also shown that phytobiotic extracts can be effective alternatives to antimicrobial growth promoters.

Acknowledgements

R. Hermes was supported by an AGAUR Doctoral Scholarship from the Catalonia Ministry of Education.

Competing Interests

The authors declare that they have no competing interests.

References

[1] Dibner, J.J. and Richards, J.D. (2005) Antibiotic Growth Promoters in Agriculture: History and Mode of Action. *Poultry Science*, **84**, 634-643. http://dx.doi.org/10.1093/ps/84.4.634

[2] Niewold, T.A. (2007) The Nonantibiotic Anti-Inflammatory Effect of Antimicrobial Growth Promoters, the Real Mode of Action? A Hypothesis. *Poultry Science*, **86**, 605-609. http://dx.doi.org/10.1093/ps/86.4.605

Version postprint

Comment citer ce document :
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

[3] Kalmokoff, M., Waddington, L.M., Thomas, M., Liang, K.L., Ma, C., Topp, E., Dandurand, U.D., Letellier, A., Matias, F. and Brooks, S.P. (2011) Continuous Feeding of Antimicrobial Growth Promoters to Commercial Swine during the Growing/Finishing Phase Does Not Modify Faecal Community Erythromycin Resistance or Community Structure. *Journal of Applied Microbiology*, **110**, 1414-1425. http://dx.doi.org/10.1111/j.1365-2672.2011.04992.x

[4] Buret, A.G. (2010) Immuno-Modulation and Anti-Inflammatory Benefits of Antibiotics: The Example of Tilmicosin. *Canadian Journal of Veterinary Research*, **74**, 1-10.

[5] Bosi, P., Merialdi, G., Scandurra, S., Messori, S., Bardasi, L., Nisi, I., Russo, D., Casin, L. and Trevisi, P. (2011) Feed Supplemented with 3 Different Antibiotics Improved Food Intake and Decreased the Activation of the Humoral Immune Response in Healthy Weaned Pigs but Had Differing Effects on Intestinal Microbiota. *Journal of Animal Science*, **89**, 4043-4053. http://dx.doi.org/10.2527/jas.2010-3311

[6] Dunston, C.R., Griffiths, H.R., Lambert, P.A., Staddon, S. and Vernallis, A.B. (2011) Proteomic Analysis of the Anti-Inflammatory Action of Minocycline. *Proteomics*, **11**, 42-51. http://dx.doi.org/10.1002/pmic.201000273

[7] Lillehoj, H.S. and Lee, K.W. (2012) Immune Modulation of Innate Immunity as Alternatives-to-Antibiotics Strategies to Mitigate the Use of Drugs in Poultry Production. *Poultry Science*, **91**, 1286-1291. http://dx.doi.org/10.3382/ps.2012-02374

[8] Bermudez-Brito, M., Plaza-Díaz, J., Fontana, L., Muñoz-Quezada, S. and Gil, A. (2013) *In Vitro* Cell and Tissue Models for Studying Host-Microbe Interactions: A Review. *British Journal of Nutrition*, **109**, S27-S34. http://dx.doi.org/10.1017/s0007114512004023

[9] Elinav, E., Henao-Mejia, J. and Flavell, R.A. (2013) Integrative Inflammasome Activity in the Regulation of Intestinal Mucosal Immune Responses. *Mucosal Immunology*, **6**, 4-13. http://dx.doi.org/10.1038/mi.2012.115

[10] Khadem, A., Soler, L., Everaert, N. and Niewold, T.A. (2014) Growth Promotion in Broilers by Both Oxytetracycline and *Macleaya cordata* Extract Is Based on Their Anti-Inflammatory Properties. *British Journal of Nutrition*, **112**, 1110-1118. http://dx.doi.org/10.1017/S0007114514001871

[11] Botting, R.M. (2010) Vane's Discovery of the Mechanism of Action of Aspirin Changed Our Understanding of Its Clinical Pharmacology. *Pharmacological Reports*, **62**, 518-525. http://dx.doi.org/10.1016/S1734-1140(10)70308-X

[12] Xu, Z.R., Kornegay, E.T., Sweet, L.A., Lindemann, M.D., Veit, H.P. and Watkins, B.A. (1990) Effects of Feeding Aspirin and Soybean Oil to Weanling Pigs. *Journal of Animal Science*, **68**, 1639-1647.

[13] Berberov, E.M., Zhou, Y., Francis, D.H., Scott, M.A., Kachman, S.D. and Moxley, R.A. (2004) Relative Importance of Heat-Labile Enterotoxin in the Causation of Severe Diarrheal Disease in the Gnotobiotic Piglet Model by a Strain of Enterotoxigenic *Escherichia coli* That Produces Multiple Enterotoxins. *Infection and Immunity*, **72**, 3914-3924. http://dx.doi.org/10.1128/IAI.72.7.3914-3924.2004

[14] Hermes, R.G., Manzanilla, E.G., Martín-Orúe, S.M., Pérez, J.F. and Klasing, K.C. (2011) Influence of Dietary Ingredients on *in Vitro* Inflammatory Response of Intestinal Porcine Epithelial Cells Challenged by an Enterotoxigenic *Escherichia coli* (K88). *Comparative Immunology, Microbiology and Infectious Diseases*, **34**, 479-488. http://dx.doi.org/10.1016/j.cimid.2011.08.006

[15] Arce, C., Ramírez-Boo, M., Lucena, C. and Garrido, J.J. (2010) Innate Immune Activation of Swine Intestinal Epithelial Cell Lines (IPEC-J2 and IPI-2I) in Response to LPS from *Salmonella typhimurium*. *Comparative Immunology, Microbiology and Infectious Diseases*, **33**, 161-174. http://dx.doi.org/10.1016/j.cimid.2008.08.003

[16] Schierack, P., Nordhoff, M., Pollmann, M., Weyrauch, K.D., Amasheh, S., Lodemann, U., Jores, J., Tachu, B., Kleta, S., Blikslager, A., Tedin, K. and Wiele, L.H. (2006) Characterization of a Porcine Intestinal Epithelial Cell Line for *in Vitro* Studies of Microbial Pathogenesis in Swine. *Histochemistry and Cell Biology*, **125**, 293-305. http://dx.doi.org/10.1007/s00418-005-0067-z

[17] Koh, S.Y., George, S., Brözel, V., Moxley, R., Francis, D. and Kaushik, R.S. (2008) Porcine Intestinal Epithelial Cell Lines as a New *in Vitro* Model for Studying Adherence and Pathogenesis of Enterotoxigenic *Escherichia coli*. *Veterinary Microbiology*, **130**, 191-197. http://dx.doi.org/10.1016/j.vetmic.2007.12.018

[18] Geens, M.M. and Niewold, T.A. (2010) Preliminary Characterization of the Transcriptional Response of the Porcine Intestinal Cell Line IPEC-J2 to Enterotoxigenic *Escherichia coli*, *Escherichia coli*, and *E. coli* Lipopolysaccharide. *Comparative and Functional Genomics*, **2010**, 469583. http://dx.doi.org/10.1155/2010/469583

[19] Van Hemert, S., Hoekman, A.J., Smits, M.A. and Rebel, J.M. (2006) Early Host Gene Expression Responses to a Salmonella Infection in the Intestine of Chickens with Different Genetic Background Examined with cDNA and Oligonucleotide Microarrays. *Comparative Biochemistry and Physiology Part D*: *Genomics and Proteomics*, **1**, 292-299. http://dx.doi.org/10.1016/j.cbd.2006.05.001

[20] Collado-Romero, M., Arce, C., Ramirez-Boo, M., Carvajal, A. and Garrido, J.J. (2010) Quantitative Analysis of the Immune Response upon *Salmonella typhimurium* Infection along the Porcine Intestinal Gut. *Veterinary Research*, **41**,

Version postprint

Comment citer ce document : Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144

23. http://dx.doi.org/10.1051/vetres/2009072

[21] Kantas, D., Papatsiros, V.G., Tassis, P.D., Athanasiou, L.V. and Tzika, E.D. (2015) The Effect of a Natural Feed Additive (*Macleaya cordata*), Containing Sanguinarine, on the Performance and Health Status of Weaning Pigs. *Animal Science Journal*, **86**, 92-98. http://dx.doi.org/10.1111/asj.12240

Version postprint

Scientific Research Publishing

Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, Linkedin, Twitter, etc
A wide selection of journals (inclusive of 9 subjects, more than 200 journals)
Providing a 24-hour high-quality service
User-friendly online submission system
Fair and swift peer-review system
Efficient typesetting and proofreading procedure
Display of the result of downloads and visits, as well as the number of cited articles
Maximum dissemination of your research work

Submit your manuscript at: http://papersubmission.scirp.org/

Comment citer ce document
Soler-Vasco, L., Hermes, R., Niewold, T. A. (Auteur de correspondance) (2016). Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences, 07 (11), 1531-1537. DOI : 10.4236/ajps.2016.711144