

Changes in the fecal profile of inflammatory markers after moderate consumption of red wine: a human trial study

Irene Muñoz-González,¹
Irene Espinosa-Martos,²
Juan M. Rodríguez,^{2,3}
Ana Jiménez-Girón,¹
Pedro J. Martín-Álvarez,¹
Begoña Bartolomé,¹
Victoria Moreno-Arribas¹

¹Instituto de Investigación Ciencias en la Alimentación, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid; ²Probisearch SL, Santiago de Compostela; ³Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Universidad Complutense Madrid, Spain

Abstract

The aim of this work was to evaluate the potential of moderate consumption of red wine to modulate the intestinal inflammation response on healthy humans. Fecal samples from a human intervention study (n=34) were collected before and after consumption of red wine for 4 weeks, and 24 immune markers including immunoglobulins, cytokines, chemokines and growth factors, were analysed. When considering the whole group of case volunteers, almost no statistically significant differences were found in the immune markers after wine consumption. However, a detailed exploration of the values differentiated a 6-volunteer subgroup that showed unusually high values of cytokines before wine consumption. For this subgroup, wine consumption significantly reduced the content of 16 out of 24 markers down to usual values, especially noticeable for cytokines related to the promotion of initial inflammation (tumor necrosis factor-alpha, interleukin 6 and interferon-gamma). This study reveals, for the first time, changes in the fecal profile of inflammatory markers after moderate consumption of red wine.

Introduction

The human immune system is responsible for protecting the body against the constant threat of antigens from the surrounding environment. Briefly, the intestinal immune response is activated when an antigen is

transported through epithelium and contact with immune cells (macrophages/monocytes and T and B lymphocytes), which release soluble immune markers such as immunoglobulins and cytokines. Cytokines are a group of low molecular weight proteins that act mediating complex interactions between the different cells of the immune system. They play an essential role in the homeostasis and regulation of the local intestinal immune response.^{1,2} Cytokines can be divided into two categories: those that up-regulate (pro-inflammatory) and those that down-regulate (anti-inflammatory) the inflammatory response. Inflammation is characterized by the interplay between pro- and anti-inflammatory cytokines,³ so an imbalance of pro-inflammatory cytokines could have an important role in the development of inflammatory bowel diseases (IBDs).^{4,5} Since any inflammatory marker produced locally as a result of intestinal inflammation may leak into the bowel lumen and be excreted in the stools, the quantification of these inflammation mediators [*e.g.* interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), and interleukin 8 (IL-8)] could be considered as an useful and non-invasive technique to detect inflammation and to ascertain the effect of dietary interventions on it.

In the last decade, a wide number of *in vitro* studies have suggested the anti-inflammatory role of wine and wine polyphenols, mainly in relation to cardiovascular diseases, and particularly related to seric immune markers.⁶⁻¹⁰ However, in relation to intestinal inflammation, only *in vitro* experiments using colonic cell models of inflammation have been performed to investigate the effects of wine-inherent polyphenols on intestinal inflammation.¹¹⁻¹³ Therefore, in this work, the effect of moderate consumption of wine on the human gut immune status was investigated. For this purpose, a wide spectrum of fecal immune parameters, including several immunoglobulins, cytokines, chemokines and growth factors, were analyzed in feces from healthy volunteers (n=34) before and after moderate consumption of red wine for 4 weeks.

Materials and Methods

Human intervention study

A randomized and controlled intervention study that consisted in the moderate consumption of a young red wine by healthy volunteers (n=42, 34 cases and 8 controls) was performed.¹⁴ The wine used was a young Merlot red wine (Penedés, Spain) (total polyphenol content=1758 mg of gallic acid/L) kindly provided by Miguel Torres S.A. winery (Catalonia, Spain). The study was divided into 2 consecu-

Correspondence: Victoria Moreno-Arribas, Instituto de Investigación Ciencias en la Alimentación, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, C/Nicolás Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain. Tel: +34.91.0017900 - Fax: +34.91.0017905. E-mail: victoria.moreno@csic.es

Key words: red wine, human intervention, inflammatory markers, cytokines, feces.

Acknowledgments: this work was funded by the MINECO (Spanish National Projects AGL2012-40172-C02-01), CONSOLIDER INGENIO 2010 (FUN-C-FOOD, CSD2007-063, Spain), and the Comunidad de Madrid (ALIBIRD-CM S2013/ABI-2728) project. IMG and AJG would like to thank the MINECO-FPI Program, the European Social Fund and JAE-Doc Program (CSIC) for their research contracts.

Received for publication: 31 October 2014.
Revision received: 1 December 2014.
Accepted for publication: 4 December 2014.

This work is licensed under a Creative Commons Attribution 3.0 License (by-nc 3.0).

©Copyright I. Muñoz-González et al., 2014
Licensee PAGEPress, Italy
Wine Studies 2014; 3:4815
doi:10.4081/ws.2014.4815

tive periods: a washout period (14 days) followed by a 28 days period during which the volunteers drank 250 mL/day of red wine, divided into 2 doses. During both periods, participants maintained the restriction of drinking any other alcoholic beverage and followed a low-polyphenols diet. The control group (n=8) followed the same pattern as the case group (n=34), with the exception that no wine was ingested during this 4-week period. Feces were immediately frozen and stored at -80°C awaiting analysis. The study was carried out according to the rules and approval of the Bioethics committee.

Preparation of fecal solutions

Fecal samples (before and after intervention) were thawed at room temperature and weighted (0.1 g). Then, 1 mL of sterile phosphate buffer saline was added and solutions vortexed and centrifuged (15 min, 14,000 rpm, 4°C). Supernatants (~200 L) were collected and used for the Multiplex Human Isotyping assay.

Detection and quantification of immune compounds in the fecal samples

Fecal concentration of immunoglobulins (Ig) (IgG1, IgG2, IgG3, IgG4, IgM and IgA) and cytokines, chemokines and growth factors,

including interleukins (IL) (IL-1_β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, and IL-17), IFN- γ , TNF- α , growth-regulated oncogene-alpha (Gro- α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1_β (MIP-1_β), granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) were determined in the fecal solutions according to the methodology of Muñoz-González *et al.*¹⁴ The Bio-Plex Pro Human Isotyping assay and the human cytokine group I and II assay kits (Bio-Rad Laboratories, Hercules, CA, USA) were used following the manufacturer's protocol, in a Bioplex 200 system instrument (Bio-Rad Laboratories). Sample determinations were carried out in duplicate. Standard curves of each analyte were performed from triplicates of each assayed concentration on each plate, with the Bio-Plex Manager 6.0 software (Bio-Rad Laboratories). The concentration of IgA in the fecal solutions was determined using a specific enzyme-linked immunosorbent assay (ELISA) kit (Cusabio Biotech Co., LTD., Wuhan, China), according to the manufacturer's instructions.

Statistical analysis

The statistical analysis was performed using STATISTICA software. As the data were not

normally distributed, medians and interquartile ranges (Q1 and Q3) were calculated for all groups. Principal components analysis (PCA) was used for exploration of the data and tentative classification of the case volunteers. For assessing significant differences between low- and high-cytokine-values subgroups before intervention, the nonparametric Mann-Whitney U test was performed. For assessing significant differences before and after the intervention period for any group/subgroup, the nonparametric Wilcoxon test was applied. In both cases, $P < 0.05$ were considered to be significant.

Results

The preliminary analysis of the data revealed a high inter-individual variability in the fecal concentration of the immune compounds analyzed ($n=24$), both before and after the wine intervention (data not shown). When all the volunteers who consumed wine ($n=34$) were considered globally, no significant differences in any fecal immune parameter (immunoglobulins, cytokines, chemokines and growth factors) were observed between samples before and after wine consumption, except for IL-13 ($P=0.044$). Interestingly, a

detailed exploration of the data indicated that a group of 6 volunteers showed unusual high values of cytokines (*i.e.*, 120.9-3624 ng/g for IFN- γ). This was supported by the PCA analysis that showed that these 6 volunteers were clearly separated from the rest according to the PC1 component (60.7% of the total variance explained) that was correlated to the concentration of cytokines. As a consequence, these six volunteers exhibiting high fecal cytokine values before intervention were taken as a subgroup (high-cytokines-values subgroup) clearly differentiated from the rest (low-cytokines-values subgroup, $n=28$) of the intervention group. For the first subgroup, statistically significant differences were found in 16 out of 24 immune markers (Figure 1). The higher changes were found for cytokines TNF, IL-6 and IFN, with 90-, 58- and 30-fold reductions, respectively, after wine intake. These cytokines are considered as pro-inflammatory cytokines and are particularly related to acute phases of inflammation. On the contrary, for the second subgroup (low-cytokine-values subgroup, $n=28$), wine consumption did not lead to significant changes in the fecal inflammatory content, except for IL-13 (Figure 2). Finally, for the control group, no significant differences were observed for any fecal immune parameter (immunoglobulins, cytokines, chemokines and growth factors) after the intervention period (data not shown).

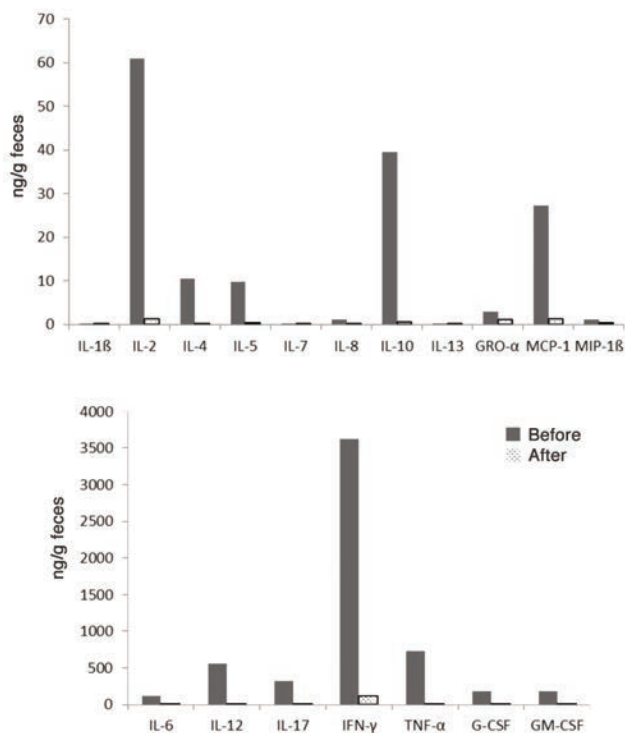


Figure 1. Representation of the median values (ng/g feces) of the cytokines, chemokines and growth factors analyzed in the fecal samples provided by the high-cytokine values subgroup ($n=6$).

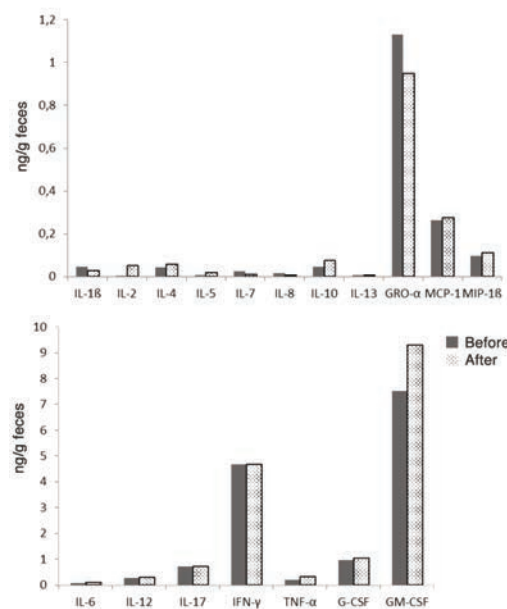


Figure 2. Representation of the median values (ng/g feces) of the cytokines, chemokines and growth factors analyzed in the fecal samples provided by the low-cytokine values subgroup ($n=28$).

Discussion

The anti-inflammatory *in vivo* effect of wine intake has been only studied in relation to cardiovascular diseases and particularly related to seric immune markers. For example, Chiva-Blanch *et al.*⁹ studied the effect of a red wine, a red wine extract and a gin on a male population with high cardiovascular risk, and Sacanella *et al.*¹⁰ studied the effect of red and white wine on healthy women. Both studies reported a reduction in the concentration of some cardiovascular-related immune markers such as IL-6 and MCP-1 after wine intake.

Our results revealed, for the first time, an important decrease in the fecal content of inflammatory markers after moderate consumption of red wine, for those volunteers showing unusually high basal values of cytokines. Changes were especially noticeable for those cytokines that promote initial inflammation (TNF- α , IL-6 and IFN- γ). This could have a beneficial effect on the intestinal immunomodulation and inflammation in healthy volunteers. The great number of immune markers analyzed permitted to have a complete overview of the immune status of the volunteers.

Cytokines play a key role in regulating the intestinal immune response and inflammation which is characterized by the loss of the physiological equilibrium between pro- and anti-inflammatory cytokines.³ It is worth to remark that elevated values of fecal pro-inflammatory cytokines have been related to the development of IBDs and high levels of cytokines, mainly TNF- α , IL-1 $_g$ and IL-6 have been found in feces of IBD patients.¹⁵ Previous studies with colonic cells models have shown anti-inflammatory effects of wine extracts.^{1,2} These studies have demonstrated significant reductions of cytokines, such as IL-6, IL-8 and TNF- α , when application of red wine extracts to induced-inflammation-colonic cells models. Others authors have tested the anti-inflammatory efficacy of individual wine phenolic compounds [malvidin, genistein and (-)-epigallocatechin-3-gallate], which promoted decreases in some cytokines, such as IL-6 or IL-8.^{11,16}

Additionally, few *in vivo* studies with rats^{17,18} have found significant reductions in the expression of IL-1 $_g$, IL-8, IFN- γ and TNF- α , or IL-1 $_g$ and TNF- α , respectively, when administering hydrocaffeic acid or resveratrol, respectively, to the dextran sulphate sodium-treated rats used as a colitis model. Although most of these studies were conducted with the original phenolic forms present in wine or food, their derived metabolites may exhibit similar or enhanced anti-inflammatory properties.¹⁷

The wine used in this study was especially rich in flavan-3-ols (270 mg/L),¹⁴ one of the most abundant group of phenolic compounds

present in wine. In a previous work, we observed that moderate consumption of this red wine promoted a significant increase in the content of phenolic metabolites (including benzoic, mandelic, hippuric, phenylacetic, phenylpropionic, cinnamic and valeric acids, phenols, valerolactones, and others) in feces.¹⁴ Therefore, the decrease observed in several cytokines after wine intake for the high-cytokine-values subgroup (n=6) might be related to the increase in the fecal content of phenolic metabolites with potential anti-inflammatory properties derived from the metabolism of wine polyphenols. Currently, we are carrying out further statistical analysis to explore possible relationships between contents of immune parameters and contents of phenolic metabolites present in feces as a consequence of wine consumption.¹⁹

Conclusions

In conclusion, this study with healthy volunteers showed, for the first time, that moderate consumption of a phenolic-rich red wine can favorably modulate the immune intestinal response *in vivo* by inducing gut anti-inflammatory effects in hosts with an asymptomatic gut inflammation.

References

- Romier-Crouzet B, Van De Walle J, During A, et al. Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food Chem Toxicol* 2009;47:1221-30.
- Angel-Morales G, Noratto G, Mertens-Talcott S. Red wine polyphenolics reduce the expression of inflammation markers in human colon-derived CCD-18Co myofibroblast cells: potential role of microRNA-126. *Food Funct* 2012;3:745-52.
- Cavaillon J. Pro- versus anti-inflammatory cytokines: myth or reality. *Cell Mol Biol* 2001;47:695-702.
- Ardizzone S, Porro G. Biologic therapy for inflammatory bowel disease. *Drugs* 2005;65:2253-86.
- Wall G, Heyneman C, Pfanner T. Medical options for treating Crohn's disease in adults: focus on antitumor necrosis factor- α chimeric monoclonal antibody. *Pharmacotherapy* 1999;19:1138-52.
- Avellone G, Di Garbo V, Campisi D, et al. Effects of moderate Sicilian red wine consumption on inflammatory biomarkers of atherosclerosis. *Eur J Clin Nutr* 2006;60:41-7.
- Li H, Foerstermann U. Red wine and cardio-

vascular health. *Circ Res* 2012;111:959-61.

- Chiva-Blanch G, Arranz S, Lamuela-Raventos RM, Estruch R. Effects of wine, alcohol and polyphenols on cardiovascular disease risk factors: evidences from human studies. *Alcohol Alcoholism* 2013;48:270-7.
- Chiva-Blanch G, Urpi-Sarda M, Llorach R, et al. Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial. *Am J Clin Nutr* 2012;95:326-34.
- Sacanella E, Vazquez-Agell M, Mena MP, et al. Down-regulation of adhesion molecules and other inflammatory biomarkers after moderate wine consumption in healthy women: a randomized trial. *Am J Clin Nutr* 2007;86:1463-9.
- Bognar E, Sarszegi Z, Szabo A, et al. Antioxidant and anti-inflammatory effects in RAW264.7 macrophages of malvidin, a major red wine polyphenol. *PLoS One* 2013;8:e65355.
- Biasi F, Guina T, Maina M, et al. Phenolic compounds present in Sardinian wine extracts protect against the production of inflammatory cytokines induced by oxysterols in CaCo-2 human enterocyte-like cells. *Biochem Pharmacol* 2013;86:138-45.
- Nunes C, Ferreira E, Freitas V, et al. Intestinal anti-inflammatory activity of red wine extract: unveiling the mechanisms in colonic epithelial cells. *Food Funct* 2013;4:373-83.
- Muñoz-Gonzalez I, Jimenez-Girón A, Martín-Alvarez PJ, et al. Profiling of microbial-derived phenolic metabolites in human feces after moderate red wine intake. *J Agr Food Chem* 2013;61:9470-9.
- Nikolaus S, Raedler A, Schreiber S. Immunosenescence of the GI immune system: increased secretion of proinflammatory cytokines by LPMNC. *Gastroenterology* 1998;114:A897.
- Sergent T, Piront N, Meurice J, et al. Anti-inflammatory effects of dietary phenolic compounds in an *in vitro* model of inflamed human intestinal epithelium. *Chem Biol Interact* 2010;188:659-67.
- Larrosa M, Luceri C, Vivoli E, et al. Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Mol Nutr Food Res* 2009;53:1044-54.
- Sanchez-Fidalgo S, Cardeno A, Villegas I, et al. Dietary supplementation of resveratrol attenuates chronic colonic inflammation in mice. *Eur J Pharmacol* 2010;633:78-84.
- Muñoz-González I, Espinosa-Martos I, Rodríguez JM, et al. Moderate consumption of red wine can modulate human intestinal inflammatory response. *J Agr Food Chem* 2014;62:10567-75.