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

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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 (Penedès, 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 consecutive 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 -80ºC 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, 4ºC). 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,

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

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including interleukins (IL) (IL-1$_\beta$, 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$_\beta$ (MIP-1$_\beta$), 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).

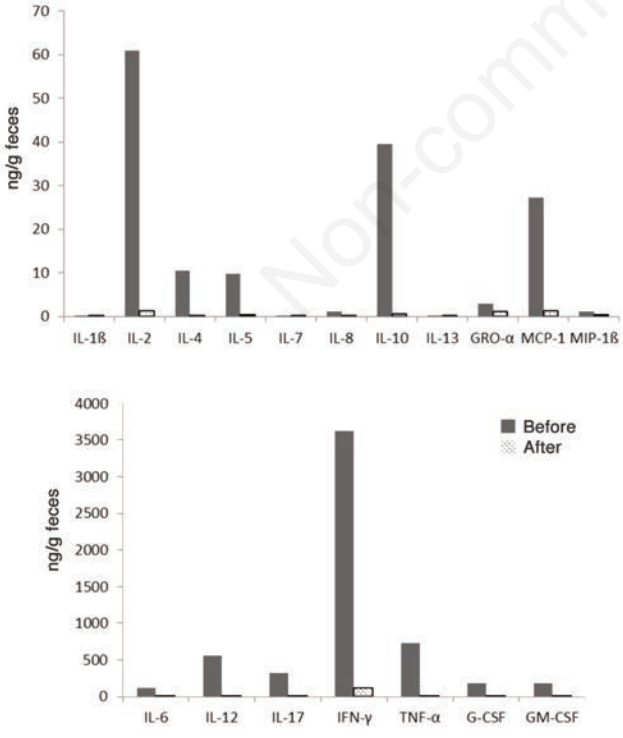

Figure 1. Representation of the median values (ng/g feces) of the cytokines, chemokines and growth factors analyzed in the fecal samples provided by the high-cytokine values subgroup (n=6).

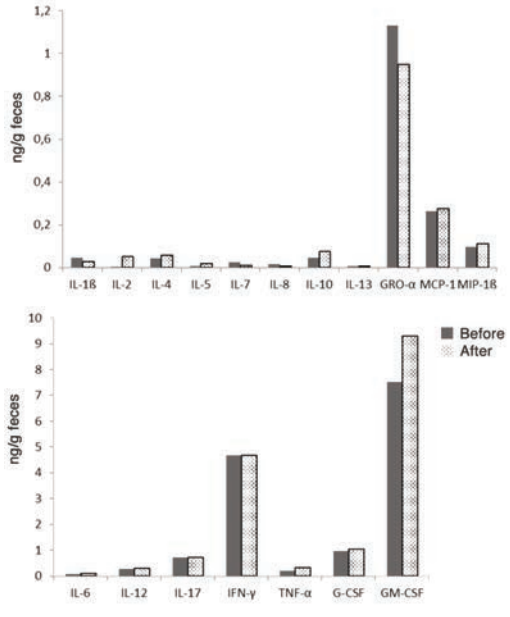

Figure 2. Representation of the median values (ng/g feces) of the cytokines, chemokines and growth factors analyzed in the fecal samples provided by the low-cytokine values subgroup (n=28).

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-a, 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 groups 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.

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