MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21:2305-2315
DOI: 10.12659/MSM.893865

Received: 2015.02.13
Accepted: 2015.04.20
Published: 2015.08.07

Combination of Azathioprine and Aminosalicylate Treatment Prevent Risk of Cardiovascular Disease in Women with Ulcerative Colitis by Reducing Inflammation

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Source of support: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPEMIG (Fundação de Amparo à pesquisa de Minas Gerais) and PRPq (Pro-Reitoria de Pesquisa)-UFMG

Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease with involvement of the immune system. Chronic inflammatory diseases have been associated with increased risk of cardiovascular disease (CVD) but few studies have assessed this risk in patients with UC and the influence of drug treatment. Thus, we evaluated the risk of development of CVD in women with UC in clinical remission, considering the drug treatment.

Material/Methods: Twenty-one women with UC participated in this study: 12 used aminosalicylates (ASA group) and 9 used azathioprine added to aminosalicylates (AZA+ASA group). The healthy control group was matched for age. We evaluated blood pressure, body composition, and biochemical and immunological parameters.

Results: Compared to the respective control group, the UC groups showed expansion of body fat and less lean body mass. Blood pressure, pro-inflammatory cytokines, nitric oxide, C reactive protein, erythrocyte sedimentation rate (ESR), and anti-oxidized LDL antibodies were higher in UC groups. Only AZA+ASA group showed increased anti-inflammatory cytokines (IL-10 and TGF-β). Framingham scores showed higher risk of CVD in UC groups. UC groups were compared and women treated with azathioprine showed reduction of total protein, globulin, ESR, and lymphocytes, with increased IL-6, TNF, IL-10, and TGF-β.

Conclusions: Our data suggest that women with UC in clinical remission have a higher risk for development of atherosclerosis and CVD when compared to the control group, while women treated with azathioprine seem more protected than those treated only with aminosalicylates, due to better regulation of the inflammatory process.

MeSH Keywords: Adipose Tissue • Aminosalicylates • Azathioprine • Cardiovascular Diseases • Cytokines • Ulcerative Colitis

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/893865

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Background

Ulcerative Colitis (UC) is an inflammatory bowel disease (IBD) whose etiology is only partially known. It is characterized by diffuse inflammation of the colonic mucosa, which starts in the rectum and extends continuously along the colon [1]. It is presented in variable clinical course with periods of remission or activity. In general, patients with UC have increased body fat and decreased lean body mass, increased production of inflammatory cytokines and they maintain a chronic low-grade inflammation [2–4]. Treatment of UC aims to induce and maintain remission of the active disease [1].

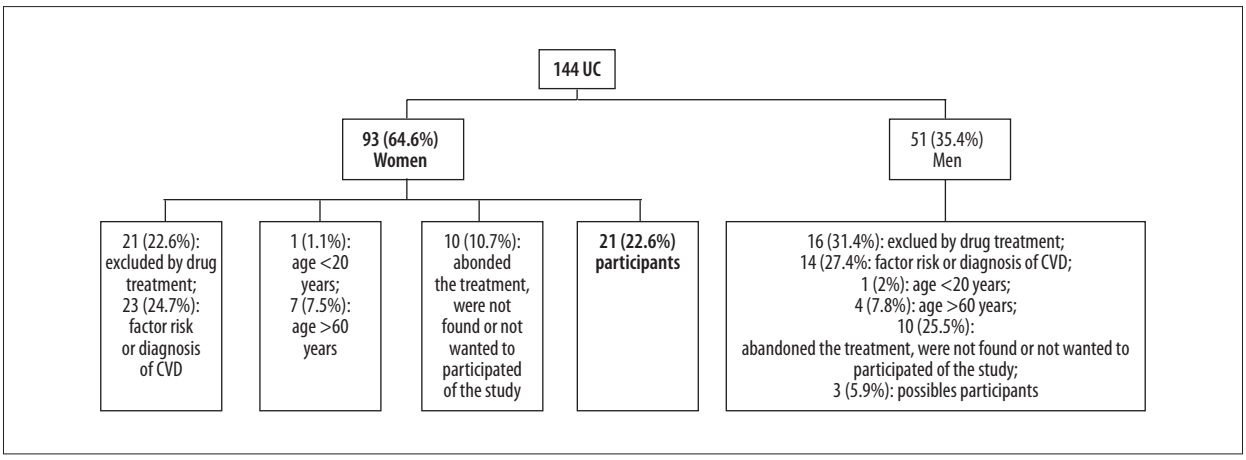
Cardiovascular diseases (CVD) are also chronic disorders associated with (low-grade) inflammation. Atherosclerosis is the main cause of CVD and is associated with endothelial dysfunction, increased accumulation of oxidized LDL (oxLDL), leukocytes, and smooth muscle cells in the intima of the artery, with release of inflammatory mediators (TNF, IL-6, CCL-2), resulting in the development and expansion of the atheroma plaque. Risk factors for CVD include smoking, alcohol, sedentary, diet, hypertension, and diabetes mellitus (DM) and dyslipidemia, as well as emerging factors such as total leukocytes count, levels of hemoglobin, and reactive C protein, IL-6, and fibrinogen [5–8].

Several studies have demonstrated that patients with chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus have higher incidence of cardiovascular disease (CVD) due to chronic and systemic inflammation [9–12]. Several studies have suggested that patients with IBD also have higher risk of atherosclerosis and cardiovascular events compared the healthy population [7,13–19]. However, there are no studies evaluating this risk in UC patients when the inflammatory process is controlled. The aim of this study was to evaluate the risk of CVD in women with UC, in clinical remission, using of aminosalicylates or azathioprine and aminosalicylates and to compare the results to those obtained in healthy matched volunteers and suggest a mechanism explaining the relationship between UC and CVD.

Material and Methods

The patients were selected at the Reference Center for Inflammatory Bowel Disease of the ALFA Institute of Gastroenterology, Clinical Hospital, Federal University of Minas Gerais (UFMG). Inclusion criteria included women aged 18–60 years that did not present hypertension, dyslipidemia, diabetes mellitus, hormonal disorders, or cardiovascular disease. Those presenting symptoms and signs of disease activity or using glucocorticoids or biologic agents were excluded. From 144 patients with UC monitored in the Reference Center, only 21 met the inclusion criteria, showing the high prevalence of risk factors for cardiovascular disease in this population and the presence of active disease phase (Supplementary Figure 1).

UC was diagnosed by clinical, laboratory, endoscopic, and histological criteria. All participants were in clinical remission [do not fit the Truelove and Witts [20] criteria] by the use of aminosalicylates (sulfasalazine or mesalazine) (12 patients) or azathioprine combined with aminosalicylate (9 patients). Because the introduction of azathioprine could modify the immunological and inflammatory status of patients, we initially divided patients in an ASA group (aminosalicylates) and an AZA+ASA



Supplementary Figure 1. Patients with UC treated in the Reference Center for Inflammatory Bowel Disease of the ALFA Institute of Gastroenterology. Of the total of 144 patients with UC treated at the Reference Center, 93 were women and 51 were men. Among men, 48 did not meet the inclusion criteria. Among women, 21 (22.6%) were excluded for drug treatment (use of corticosteroids or biology therapy); 23 (24.7%) were excluded for risk factors or diagnosis of CVD or other diseases; 8 (8.6%) were excluded by age; 10 (10.7%) abandoned the treatment, were not found, or did not want to participate in the study, and only 21 (22.6%) women met the study criteria and wanted to participate.

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group (azathioprine + aminosalicylates) to determine the inflammatory profile and cardiovascular risk; later, we compared both treatments for remission to observe possible differences. The control group consisted of by healthy age-matched women. The study was approved by the Ethics Committee in Human Research of the Federal University of Minas Gerais (protocol 342/11). All participants read and signed the informed consent.

Participants answered questionnaires about their clinical and family histories and lifestyle routines. Blood pressure and anthropometric data such as weight, height, body mass index (BMI), waist circumference, and body composition (body fat and body fat-free) were also obtained. After 12 h of fasting, 20 mL blood was collected for biochemical and inflammatory markers analysis. Total cholesterol, HDL-cholesterol, glycemia, total protein, albumin, hemoglobin, fibrinogen, and high-sensitive C reactive Protein (hs CRP) were assessed using commercial kits (Labstest® and Bioclin, Belo Horizonte, Brazil). The erythrocyte sedimentation rate (ESR) was measured using a Westergren pipette. LDL-cholesterol (LDLc) was calculated using the Friedewald equation. Globulin was determined as the difference between the total protein and albumin concentrations. Total and differential leukocyte count was obtained using a Neubauer chamber, and a differential count was performed on slides using a Rapid Kit Panoptic® kit (Laborclin, Paraná, Brazil). Nitric oxide (NO) concentration was measured indirectly according to Griess technique [21]. IL-6, TNF, IL-1β, IL-10, TGF-β, CCL-2, and anti-oxLDL antibodies were determined by ELISA (BD OptEIA ®, United States) [22,23]. The 10- and 30-year Framingham risk scores (FRS) were determined using online calculators (*http://www.framinghamheartstudy.org/*), considering lipid profile or BMI.

Data are presented as mean ± standard error or median. The Kolmogorov-Smirnov test was used to evaluate the distribution of the data. Unpaired Mann-Whitney, Wilcoxon, or Student's t-tests were used for continuous variables. Categorical variables were analyzed using Fisher's exact test. The correlation between variables was analyzed using Pearson correlation coefficient. Statistical analysis was performed using SPSS software *(Statistical Package for Social Sciences)* version 19.0. The level of significance was set at $p<0.05$.

Results

Comparison between patients with UC in clinical remission in use of aminosalicylates (ASA) and control healthy (CT)

Body weight, BMI, waist circumference, and traditional risk factors for CVD such as glycemia, triglyceridemia, total cholesterol, and its fractions were similar between groups (Tables 1, 2), while body fat was increased and fat-free mass was reduced in ASA group (Table 1). ASA patients also showed increased total proteins due to increase in globulin, with the concomitant decreased on albumin fraction (Figure 1). Diastolic and systolic blood pressures another important risk factor for CVD, and hsCRP and ESR were higher in ASA patients (Table 1, Figure 1). Inflammatory markers such as IL-6 and CCL2 were also increased in the ASA group (Figure 2). Nitrite concentration (indicating nitric oxide status) and IgG anti-oxLDL (an indicator of oxLDL concentration) were increased in women in the ASA group (Figure 3), suggesting an increased risk of atherosclerosis in these patients. The Framingham scores showed that ASA patients have a higher 10-year risk for CVD, considering lipid profile or BMI. Similar results were obtained when assessing the 30-year risk of CVD, showing higher relative risk of CVD in the ASA group (Figure 4). Together, changes in blood pressure and inflammation markers show that patients with UC in clinical remission and treated with aminosalicylates have higher risk of CVD when compared to healthy subjects.

Comparison between patients with UC in clinical remission receiving azathioprine and aminosalicylate (AZA+ASA) and healthy controls (CT)

Patients using of AZA+ASA had higher BMI (p=0.06), increased waist circumference, fat mass, and blood pressure, but there was no relation with physical activity in their routines (Table 1). Lipid profile, glycemia, total protein, globulin, albumin, hemoglobin, ESR, and fibrinogen were similar between groups (Figure 1, Table 2) and the CRP was higher in the AZA+ASA group (Figure 1). The global and differential leukocyte counts were similar between groups (Table 2). Levels of IL-6, TNF, CCL-2, IL-10, TGF-β (Figure 2), nitrite, and IgG anti-oxLDL (Figure 3) were increased in the AZA+ASA group, showing higher risk of atherosclerosis and CVD in this group. Simultaneously, adding azathioprine treatment promotes the increase of anti-inflammatory mediators in attempting to regulate the inflammatory process.

The relative risk for CVD assessed by 10- and 30-year Framingham scores was higher in the AZA+ASA group (Figure 4), suggesting increased relative risk of CVD in these patients.

Comparison between patients in use of aminosalicylates (ASA) and azathioprine combined with aminosalicylate (AZA+ASA) for UC remission

After comparing the patients with their respective controls, the remission treatments (ASA and AZA+ASA) were compared. Anthropometric data were similar between groups (Table 1). Women in the AZA+ASA group presented decreased levels of total protein, globulin, ESR, and lymphocytes (Figure 1, Table 2) compared to the ASA group, suggesting better regulation of the inflammatory process characteristic of the disease. Patients in the AZA+ASA group had higher levels of TNF, IL-6, IL-10, and

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Table 1. General characteristics of women using aminosalicilate (ASA) or azathioprine and aminosalicilate (AZA+ASA) for UC clinical remission and their respective matched controls.

Parameter	Control (ASA)	ASA	Control (AZA+ASA)	AZA+ASA
Number of patients	12	12[1]	9	9[1]
Age (years)	37.8±2.4	37.8±2.21	37.8±2.8	37.8±3.4
Time of UC diagnose	–	9.5±1.7	–	6.4±1.6
Smokers	0	0	0	0
Ethilists	9 (75%)	0[2]*	7 (77.8%)	0[2]*
Physical activity	9 (75%)	4 (33.3%)[2]	7 (77.8%)	0[2]*
Use of medication				
Sulphasalazine	–	10 (83.3%)	–	2 (22.2%)
Mesalazine	–	2 (16.7%)	–	6 (66.7%)
Azathioprine	–	0	–	9 (100%)
CVD familial history	4 (38.5%)	8 (66,7%)[2]	5 (55.5%)	4 (44,4%)[2]
Body Weight (kg)	58.4±1.7	60.2±3.7[1]	58.3±1.7	72.1±6.7[1]
BMI (kg/m^2)	22.7±0.6	23.4±1.8[3]	22.7±0.8	28.2±2.1[1]
Waist circumference (cm)	73.0±1.8	80.0±3.8[1]	73.3±2.1	89.1±5.9[1]*
Body fat (%)	29.0±1.8	37.2±2.7[1]*	29.3±2.2	36.9±2.8[1]*
Body fat free mass (kg)	42.2±1.2	36.9±1.2[1]*	41.1±1.1	41.6±2.0[3]
SBP (mmHg)	99.7±2.5	113.2±3.9[1]*	99.8±3.7	116.5±7.6[1]*
DBP (mmHg)	65.0±1.1	76.5±3.1[1]*	67.0±2.9	76.6±6.2[1]*

[1] T paired test; [2] Fisher test; [3] Mann Whitney test. * Represents differences between UC group and their respective matched control. No differences were observed between both UC groups. CVD – cardiovascular disease, BMI – Body Mass Index.

Table 2. Seric profile of the women using aminosalicilate (ASA) or azathioprine and aminosalicilate (AZA+ASA) for UC clinical remission and their respective matched controls.

Parameters	Control ASA	ASA	Control AZA+ASA	AZA+ASA
Triglycerides (mg/dL)	87.6±7.6	84.3±15.3[2]	96.2±13.7	84.3±14.0[1]
Total cholesterol (mg/dL)	184±8.0	190.2±6.8[2]	185.4±13.0	185.5±13.5[2]
HDL (mg/dL)	45.4±4.6	46.2±3.2[1]	44.2±6.1	39.6±6.1[1]
LDL (mg/dL)	126±7.7	130.2±7.2[1]	133.3±12.7	134.8±13.3[1]
Glycemia (mg/dL)	75.8±3.4	75.5±3.2[1]	72.8±3.7	81.6±3.2[1]
Hemoglobin (g/dL)	14.3±0.8	12.8±0.81[1]	13.4±0.6	14.4±1.3[1]
Total leucocyte count(cel/µL)	6321±807.4	5977±847.8[1]	5519±965.2	5025±885.7[1]
Neutrophils (cel/µL)	4055±557.7	3328±703.9[1]	3436±624.8	3143±551.6[1]
Eosinophils (cel/µL)	102.5±20.4	57.5±19.8[2]	97±26.6	53.5±63.1[2]
Lymphocytes (cel/µL)	1808±234.3	1743±217.0[2]	1720±321.2	1484±335.4[1#]
Monocytes (cel/µL)	299.7±53.2	243.8±44.9[1]	193.5±44.5	194.3±60.7[2]

[1] T paired test; [2] Mann Whitney test. # Represents diferences between UC groups. No differences were observed between UC groups and their respective matched control.

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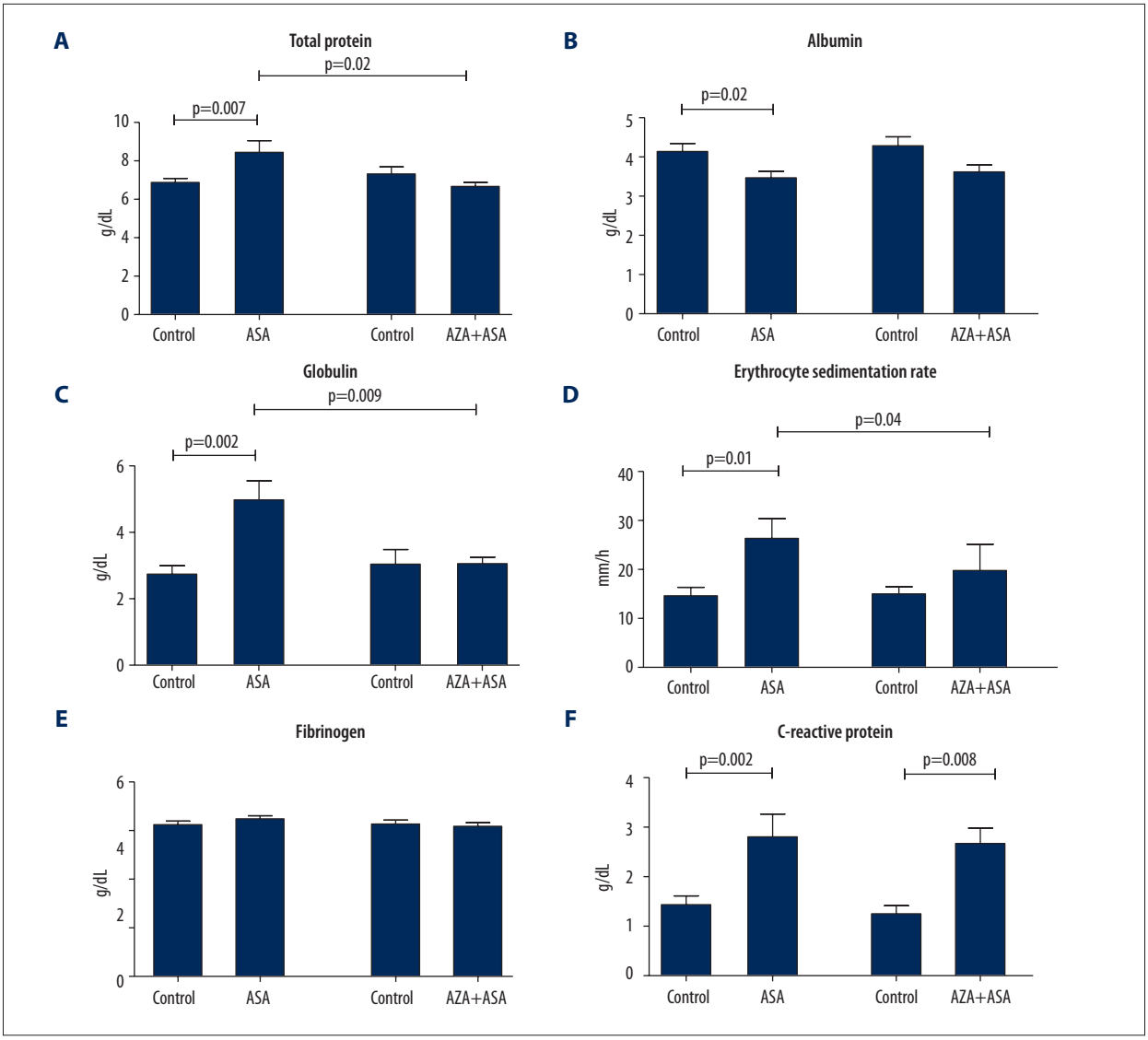

Figure 1. Markers of inflammation in women treated with aminosalicylates (ASA) or azathioprine+aminosalicylates (AZA+ASA) and their controls. (**A**) Total protein; (**B**) albumin; (**C**) globulin; (**D**) erythrocyte sedimentation rate; (**E**) fibrinogen; (**F**) C-reactive protein.

TGF-β compared to the ASA group. Others parameters were similar between groups (Tables 1, 2, Figure 3). However, the presence of these differences between both treated UC groups and the 10 and 30-year Framingham risk scores were similar between groups (Figure 4).

Discussion

In this paper we highlight the mechanisms by which patients with UC in clinical remission are at higher risk for CVD and how drug treatment influences this. This is the first work performed in patients with UC in clinical remission that associates markers of inflammation, risk of CVD, and drug treatment. Compared with healthy women, women with UC in clinical remission showed increased body fat with reduced fat-free mass. The AZA+ASA group still had an increase in BMI and waist circumference and in risk markers for CVD, including diseases such as diabetes mellitus and dyslipidemia [24]. Changes in body composition have been reported in IBD patients [3,25,26] and could be related to several factors such as poor eating habits, physical inactivity, and previous use of steroids. Furthermore, patients with Crohn's disease have increased number of adipocytes, with reduced volume and increased inflammatory infiltrate of mononuclear cells, favoring expansion and inflammatory capacity. Adipose tissue is an endocrine organ that produces and secretes inflammatory cytokines and nitric oxide, leading to a chronic low-grade systemic inflammation, increasing the

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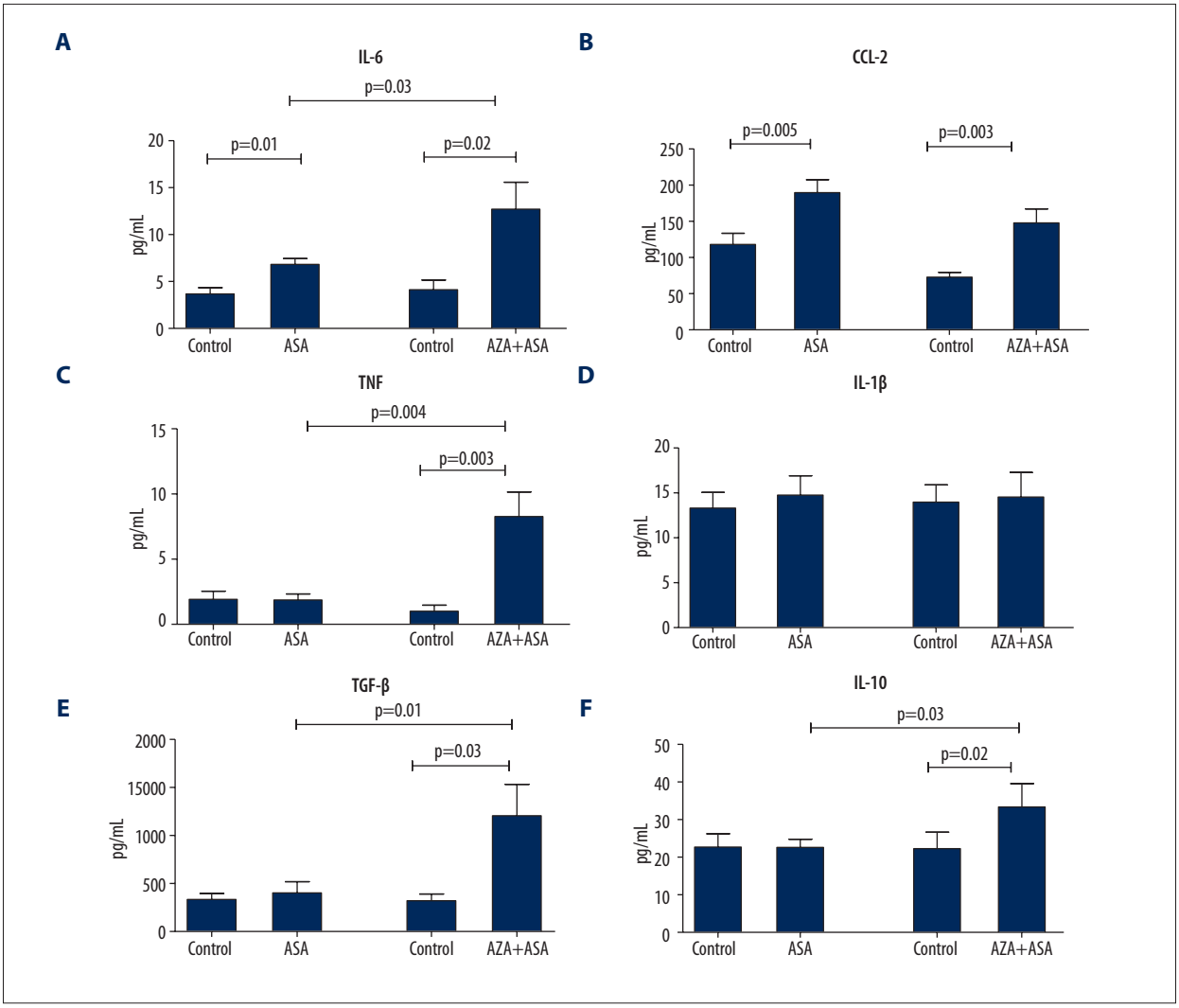

Figure 2. Serum levels of cytokines in women treated with aminosalicylates (ASA) or azathioprine+aminosalicylates (AZA+ASA) and their controls. (**A**) Interleukin-6 – IL-6; (**B**) Chemokine (CC motif) ligand 2 – CCL-2; (**C**) Tumor necrosis factor – TNF; (**D**) Interleukin-1β – IL-1β; (**E**) Transforming growth factor beta – TGF-β; (**F**) Interleukin-10 – IL-10.

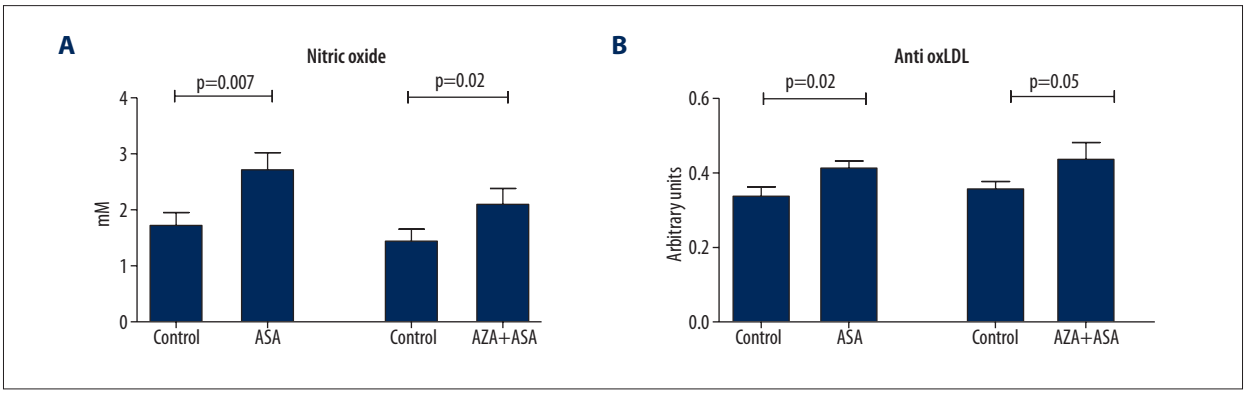

Figure 3. Serum markers of oxidative stress in women treated with aminosalicylates (ASA) or azathioprine+aminosalicylates (AZA+ASA) and their controls. (**A**) nitric oxide; (**B**) anti-oxidized LDL.

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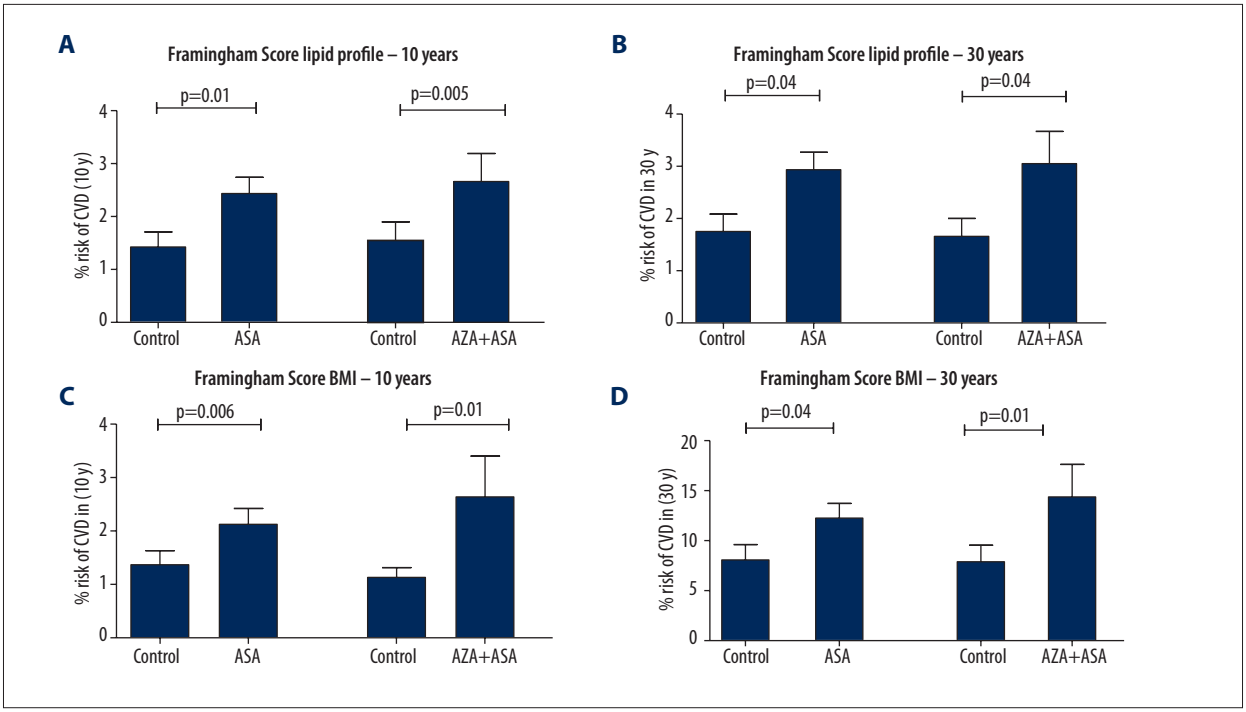

Figure 4. Risk of cardiovascular disease at 10 and 30 years in women treated with aminosalicylates (ASA) or azathioprine+aminosalicylates (AZA+ASA) and their controls. The Framingham Risk Score for CVD over a period of 10 years (**A**) or 30 years (**B**) according to the lipid profile; CVD risk over a period of 10 years (**C**) or 30 years (**D**) according to BMI.

risk for CVD. The expansion of adipose tissue may contribute to increased blood pressure, another risk factor for CVD that has been observed in IBD [13] and in this study. Even in remission, patients with UC have a subclinical inflammatory response in the colon, which may also contribute to the increase of these inflammatory mediators.

The reduction in fat-free mass in the UC group can indicate changes in muscle mass, bone mass, and hydration status and appears to be due to poor dietary habits, physical inactivity, previous use of corticosteroids, or even due to catabolic process in common chronic diseases. The increased levels of circulating cytokines and globulins (IgG anti-oxLDL and anti-cytokine antibodies) favors the diversion of amino acids for production of inflammatory mediators affecting protein synthesis and body composition. The increase of total protein seen in the ASA group is the result of increased globulins. Albumin synthesis may also be affected, leading to a reduction in its level, as seen in the ASA group [3,27,28]. Previous studies suggest the increase of anti-immunoglobulins cytokines in patients with chronic inflammatory diseases in an attempt to combat inflammatory process [29]. In the AZA+ASA group, there were no changes in these parameters, which might be associated with better immunological and inflammatory regulation and nutritional status, which were not observed in the ASA group, suggesting the effect of azathioprine in UC remission.

CRP and ESR were within recommended levels, confirming clinical remission in UC groups. Even so, the CRP levels were higher in UC groups compared to controls, while ESR was higher only in the ASA group, suggesting better regulation of the inflammatory process in the AZA+ASA group and the effect of azathioprine in this group. Inflammatory cytokines such as TNF and IL-6 induce hepatic production of CRP, which induces expression of adhesion molecules and oxLDL uptake by macrophages, favoring the development of atherosclerosis plaque and cardiovascular events [9,10,30]. Serum nitrite concentration was higher in the UC group compared to matched controls, possibly due to higher inflammatory cytokine levels that also induce iNOS expression [31], generating NO and peroxynitrite, triggering changes in vascular endothelium, and increasing expression of adhesion molecules and vascular permeability, leading to increased oxidative stress and increased blood pressure [32–34], which are risk factors for CVD.

The lipid profile parameters met the NCEP-ATPIII criteria [35] in both groups. Although the lipid profile was similar, changes in the vascular endothelium in the UC group triggered by the increase of NO, CRP, and inflammatory cytokines may favor the migration of LDL in the arterial intima and its oxidation, contributing to formation atherosclerosis plaque. High levels of anti-IgG oxLDL antibodies were found in the UC groups. LDL oxidation generates antigenic epitopes that induce the production of anti-IgG oxLDL [10]. The increase of circulating IgG anti-oxLDL is related

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to risk of atherosclerosis and suggests that patients with UC have higher levels of oxLDL than controls and higher risk of CVD.

Compared to the control group, the ASA group showed increased serum levels of IL-6 and CCL-2, while the AZA+ASA group has higher levels of TNF, IL-6, CCL-2, IL-10, and TGF-β circulating. The increases in these cytokines may be derived from adipose tissue, colonic mucosa affected by UC, or of circulating inflammatory cells as mononuclear cells [36–38]. IL-6 plays an important role in the maintenance of chronic inflammation, preventing apoptosis of lamina propria T cells. IL-6 usually binds to its membrane receptor, interacting with the gp80 and gp130 subunits, and activating the STAT3 pathway, but sometimes IL-6 binds to its soluble receptor and that interact with gp130 on the surface of cells (especially those not expressing IL-6 receptors), leading to activation of STAT3. In turn, STAT3 promotes upregulation of anti-apoptotic genes, avoiding leukocytes elimination in intestinal mucosa, thus contributing to maintenance of inflammation and release of these mediators to the circulation, even during the remission phase [39–41]. These processes, in turn, may interfere in the cardiovascular system, favoring changes in vascular permeability and LDL oxidation, and leading to development of atherogenesis and its complications. In this regard, we question whether IL-6 could interfere with leukocyte survival in atherosclerotic plaque, contributing to its maintenance and progression. IL-6 is also capable of reducing the production of NO via eNOS, resulting in endothelial dysfunction as well as increased expression of adhesion molecules and the scavenger receptors CD36 and SR-A1 [38,42–45]. Vascular permeability can be altered by these factors, favoring the migration of monocytes to the intima of arteries and the up-take of oxLDL, contributing once again to the formation of the foam cells and progression of atherosclerosis.

CCL-2, in turn promotes the recruitment of monocytes, thereby facilitating the migration to different locations, including adipose tissue, colon, and intima of arteries, thereby contributing to the formation of atheroma [10]. Although the levels of circulating leukocytes, especially monocytes, were similar between groups, the increased concentration of CCL-2 could induce migration of monocytes, perpetuating inflammation, but keeping monocyte concentration in the normal range in the systemic circulation. TNF were increased only in the AZA+ASA group, demonstrating higher severity of the disease, which can justify the use of azathioprine in this group. In general, the addition of azathioprine occurs when the patient does not respond to corticosteroids, when the patient is dependent on corticosteroids, or when the patient does not respond to conventional treatment with aminosalicylates [1], requiring the addition of the drug. TNF induces the secretion of other inflammatory mediators such as IL-1β, PCR, and NO. In addition, it also increases adhesion molecules and causes endothelial dysfunction, increasing risk of DCV [41,46–48].

Only the AZA+ASA group showed increased production of IL-10 and TGF-β, which are anti-inflammatory cytokines. IL-10 reduces the antigen presentation and activation of inflammatory cells and reduced the activation of NF-κB as well as production of inflammatory mediators [49,50], contributing to immunology homeostasis, while TGF-β participates of the modulation of the activation of endothelial cells and Th1 cells [10,51], inducing the formation of regulatory T cells [52] and promoting mucosal intestinal healing [53]. Increased levels of these cytokines in patients using azathioprine compared to controls suggest a mechanism of immunoregulation, which was not seen when the ASA group was compared to its matched controls.

When both UC groups were compared, we noted that the AZA+ASA group showed increased IL-6, CCL-2, TNF, IL-10, and TGF-β, indicating that although there is increase of inflammatory cytokines, the association of azathioprine and aminosalicylate induces increase of the anti-inflammatory cytokines (IL-10 and TGF-β), suggesting better immunoregulation in this group, and consequently reducing the risk of atherosclerosis.

Azathioprine reduces colonic inflammation by blocking Rac1 via, resulting in apoptosis of TCD4+ lymphocytes and regulating colonic inflammation [1]. Thus, azathioprine appears to modulate T cell repertoire, reducing TCD4+ lymphocytes and preserving the IL-10 and TGF-β producer regulatory T cells, and promoting immunoregulation of the inflammation related to UC and CVD in this group. Although aminosalicylates are associated with reduction of inflammatory cytokines and oxidative stress [1], the ASA group was not able to induce anti-inflammatory mediators, suggesting that this group has a more inflammatory profile that leads to a more susceptible CVD status compared to the AZA+ASA group.

The combination of azathioprine and aminosalicylates changed the profile of immune-inflammatory response in the studied patients, regulating the inflammatory process and reducing the risk of CVD, probably because the drugs have different mechanisms of action that block different inflammatory pathways. However, it is not possible to know whether the isolated use of azathioprine induces immunoregulatory response similar to that observed or whether this effect is only seen when the drugs are combined.

The relative risk for CVD, estimated by the Framingham risk scores at 10 and 30 years, showed that ASA and AZA+ASA groups had higher CVD risk compared to their controls. Although most patients did not show traditional risk factor for CVD, higher risk was probably due to the increase in blood pressure in UC groups. Although the Framingham risk scores were similar in both UC groups, the AZA+ASA group showed a better inflammatory profile, which is not included in the Framingham score, and which could benefit these patients, improving CVD risk and its complications.

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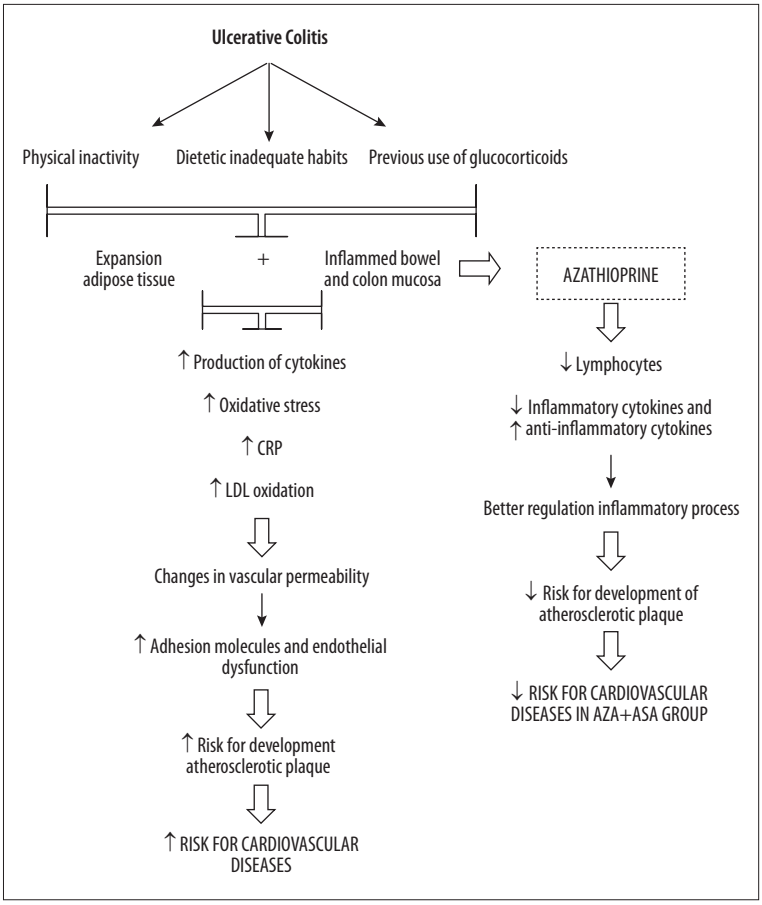

Figure 5. Schematic hypothesis of increased risk of CVD in women with UC in clinical remission. Women with UC tend to have sedentary lifestyles, inadequate dietary habits and previous use of glucocorticoids, factors that lead to increased adipose tissue. The expansion of adipose tissue and chronic inflammatory process in the colon lead to increased production of cytokines, CRP, oxidative stress, and LDL oxidation, causing alterations in vascular permeability and increased expression of adhesion molecules and endothelial dysfunction. Together, these factors accelerate the development of atherosclerosis and, consequently, a higher risk for cardiovascular diseases. In contrast, women treated with azathioprine combined with aminosalicylates showed a decrease in circulating lymphocytes and increased production of cytokines, including anti-inflammatories ones, promoting a regulatory mechanism of inflammation and reduction of risk of developing atherosclerosis and cardiovascular diseases.

We believe that previous use of glucocorticoids associated with sedentary lifestyle and poor dietary habits favor the expansion of adipose tissue. Adipose tissue expansion and the inflamed bowel mucosa are factors related to higher production of cytokines and oxidative stress. Increased inflammation markers result in changes in the vascular permeability, including increased expression of adhesion molecules and endothelial dysfunction, favoring the development of complications such as increased blood pressure and migration of monocytes to the arterial intima. Increased inflammatory cytokines, RCP, and NO also induce LDL oxidation, which was indirectly seen by anti-oxLDL antibodies. Together, this profile favors the development of atherosclerotic plaque, resulting in a higher risk for CVD. In turn, therapy for maintenance of clinical remission affects the inflammatory response, suggesting that the addition of azathioprine to conventional treatment with aminosalicylates decreases the inflammatory process, induces anti-inflammatory mediators, and reduces the risk of CVD in this group (Figure 5).

Conclusions

Women with UC in clinical remission maintain a more inflammatory profile compared to their healthy controls, suggesting increased risk of atherosclerosis and CVD. The addition of azathioprine to treatment with aminosalicylates reduces inflammation and increases anti-inflammatory cytokines, evidencing better regulation of inflammatory processes and reducing the risk of CVD in this group when compared to that treated with aminosalicylates alone.

Acknowledgments

We also thank Rosalia Carvalho Pereira and Fabiola Fernandes Fagundes for their assistance with blood collection.

Conflicts of interest

Conflicts of interest and sources of funding: there are no conflicts of interest.

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