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Original Article

Quercetin reduces markers of oxidative stress and inflammation in sarcoidosis

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SUMMARY

Background & aims: Oxidative stress and low antioxidant levels are implicated in the aetiology of sarcoidosis, an inflammatory disease. Quercetin is a potent dietary antioxidant that also displays anti-inflammatory activities. Consequently, the aim is to examine the effect of quercetin supplementation on markers of oxidative stress and inflammation in sarcoidosis.
Methods: A double-blind intervention study has been conducted with two groups of non-smoking, untreated sarcoidosis patients, matched for age and gender. One group was given 4x500 mg quercetin ($n = 12$) orally within 24 h, the other one placebo ($n = 6$). Plasma malondialdehyde levels were used as marker of oxidative damage, plasma ratios of TNFα/IL-10 and IL-8/IL-10 as pro-inflammatory markers.
Results: Quercetin supplementation improved the antioxidant defence, indicated by the increased total plasma antioxidant capacity. Moreover, quercetin supplementation also reduced markers of oxidative stress and inflammation in the blood of sarcoidosis patients. The effects of quercetin supplementation appeared to be more pronounced when the levels of the oxidative stress and inflammation markers were higher at baseline.
Conclusions: Sarcoidosis patients might benefit from the use of antioxidants, such as quercetin, to reduce the occurring oxidative stress as well as inflammation. The effects of long-term use of antioxidant supplementation in sarcoidosis, using e.g. quercetin, on improvement of lung function remain to be investigated. (www.clinicaltrials.gov; NCT-00402623).

1. Introduction

Sarcoidosis is a chronic inflammatory disease of which the exact cause still needs to be elucidated. Besides the presence of a chronic inflammatory process, sarcoidosis is also associated with the occurrence of oxidative stress, i.e. an imbalance between the production of and the protection against reactive oxygen species (ROS). This is deduced from increased levels of biomarkers of oxidative damage such as exhaled ethane[1] and both 8-isoprostane[2] and oxidized proteins[3] in the bronchoalveolar lavage fluid (BALF) of sarcoidosis patients. Recently, we have found that the total antioxidant capacity of sarcoidosis patients is approximately 75% of that of matched controls[4].

To increase the total antioxidant capacity in chronic diseases associated with enhanced oxidative stress, such as sarcoidosis, antioxidant supplementation has gained a lot of interest the past few years.[5] A good candidate for such supplementation could be the dietary antioxidant quercetin. Indeed, it has recently been shown that quercetin supplementation effectively increases both the plasma quercetin concentration and the total plasma antioxidant capacity in healthy volunteers.[6,7,8] Moreover, it is known that, within the flavonoid family, quercetin is the most active scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS) both *in vitro* and *in vivo*.[9] For example, the antioxidant capacity of quercetin is several times that of various endogenous antioxidants including glutathione and vitamin E[10]. This can be explained by the presence of two antioxidant pharmacophores within the molecule that both have the optimal configuration for free radical scavenging.[11]

Abbreviations used: BALF, bronchoalveolar lavage fluid; DLCO, diffusing capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GSH, glutathione; GSSG, glutathione disulphide; IL, interleukin; LPS, lipopolysaccharide; MDA, malondialdehyde; NF-κB, nuclear factor kappa-B; RNS, reactive nitrogen species; ROS, reactive oxygen species; SSA, sulfosalicyclic acid; TCA, trichloric acid; TEAC, trolox equivalent antioxidant capacity; TNFα, tumor necrosis factor alpha; UA, uric acid.

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Interestingly, quercetin displays more characteristics that make it an excellent candidate for antioxidant supplementation in sarcoidosis. Foremost, several studies have indicated that quercetin, both added *in vitro* and supplemented *in vivo*, also displays anti-inflammatory effects.[4,8,12] Indeed, it is known that this flavonoid is capable of reducing LPS-induced levels of various pro-inflammatory cytokines including TNFα and IL-8, two cytokines known to be elevated in sarcoidosis.[4] Secondly, de Boer et al have demonstrated that quercetin accumulates in the lungs of rats.[13] This finding suggests that the flavonoid is expected to exert its positive effects especially in this organ, which is also primarily involved in sarcoidosis. Moreover, this specific tissue distribution of quercetin correlates well with the observations of Kumar et al that oral quercetin supplementation offers protection against pulmonary damage induced by influenza virus infection in mice.[14,15]

The combination of its tissue specific distribution and potent anti-oxidative as well as anti-inflammatory capacities prompted us to study the effect of quercetin supplementation on *in vivo* markers of oxidative stress and inflammation in sarcoidosis patients. Moreover, to mimic a severe inflammatory burden that might occur by incidental exposure to e.g. dust particles, cigarette smoke or other triggers, an additional *ex vivo* LPS challenge was performed in the blood to study the protecting potential of this quercetin supplementation.

2. Materials and methods

2.1. Materials

Quercetin and lipopolysaccharide (LPS, *E. coli* 0.26:B6) were purchased from Sigma Chemical Co. (St. Louis, USA). RPMI 1640 medium containing L-glutamine was obtained from *Gibco (UK)*. Human TNFα (7300 pg/ml), human IL-10 (4000 pg/ml) and human IL-8 (10 ng/ml) were acquired from CLB/Sanquin (Amsterdam, the Netherlands). All other chemicals were of analytical grade.

3. Methods

3.1. General information

All participating patients were recruited via their own physician. All participants were fully informed, both written and orally, about the aim and details of the study and have given their written informed consent.

The study was carried out at the University Hospital Maastricht after approval of the protocol by the Medical Ethics Committee of Maastricht University and the University Hospital Maastricht and registration of the study at www.clinicaltrials.gov (NCT-00402623). Based on foodstuff questionnaires, it was concluded that all participants had comparable dietary habits with an average daily intake of quercetin of approximately 15 mg. None of the participants took any medication or vitamin or food supplements either prior to or during the study. Randomisation occurred by first dividing all participants into trios based on their age and gender and then by randomly giving placebo treatment to one individual out of each trio.

3.2. Participants

Eighteen Caucasian non-smoking patients with symptomatic sarcoidosis (age 45 ± 10, 12 male and 6 female) were enrolled. Sarcoidosis had been diagnosed based on both clinical features and bronchoalveolar lavage (BAL) fluid analysis results (data not shown).[16] Moreover, a biopsy confirmation of the disease had been performed in 11 out of the 18 sarcoidosis patients. The clinical symptoms of all patients included respiratory symptoms, i.e. dyspnea, coughing and chest pain. None of the participants suffered from extra-pulmonary involvement of sarcoidosis during the study. The characteristics of the study population are summarized in Table 1.

3.3. Lung function measurement

Lung function measurements included forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and diffuse capacity of the lung for carbon monoxide (DLCO). FEV_1 and FVC were measured with a pneumotachograph, DLCO by the single-breath method (Masterlab, Jaeger, Würzburg, Germany). Values were expressed as a percentage of those predicted based on age and gender.

3.4. Supplementation study

Prior to the actual supplementation period, participants were subjected to a two-day wash-out period. During this period, they were not allowed to consume food rich in flavonoids in general or quercetin in particular. This food included non-organic onions, apples, red wine, tea, organic and freshly pressed fruit juices, berries (e.g. blueberries and elderberries), grapes, cherries, raisins, parsley, broccoli, cabbage,green beans and tomatoes.[17] Participants also had to minimise the use of herbs and spices during this period.

The wash-out period was followed by a 24-h supplementation period during which all participants had to take 4 capsules containing either 500 mg quercetin or a placebo. The capsules were taken throughout the day, i.e. during lunch, during dinner, just before bedtime and the last during breakfast the following morning, 3 h before the second blood withdrawal. Before and after this supplementation period, venous blood samples were drawn into EDTA-containing vacutainer tubes (Vacutainer, Becton–Dickinson, Belgium) and kept on ice prior to processing which occurred within 1 h after blood collection. During supplementation, the same dietary restrictions as during the wash-out period were applied.

3.5. Preparation of the blood samples

Blood was aliquoted into eppendorfs for both the ascorbic acid and the glutathione analysis: for the former 10% trichloric acid (TCA) was added to the whole blood, whereas 1.3% sulfosalicyclic acid (SSA) in 10 mM hydrochlorous acid (HCl) was used to preserve the samples for the latter. Another aliquot of blood was used for the incubations required for the blood-based cytokine production

Table 1
Characteristics of the participants.

	Quercetin-receiving group	Placebo-receiving group
number (m/f)	12 (8/4)	6 (4/2)
age	31–69 (46 ± 3)	34–59 (44 ± 3)
length	158–186 (174 ± 3)	158–182 (175 ± 4)
weight	64–96 (81 ± 3)	69–96 (84 ± 4)
body mass index	23–32 (27 ± 1)	24–35 (28 ± 2)
time since diagnosis	1–10 (5 ± 1)	1–11 (4 ± 2)
biopsy taken	yes: 7 no: 5	yes: 4 no: 2
DLCO	55–109 (84 ± 5)	85–107 (91 ± 6)
FEV_1	36–133 (96 ± 7)	51–101 (86 ± 7)
FVC	75–128 (104 ± 5)	65–112 (97 ± 7)
chest radiograph stage 0/I/II/III/IV (n)	3/3/3/3/1	2/2/1/1/0

Controls are matched on age and gender and do therefore not significantly differ from the patients regarding these parameters. Age is expressed in year, length in cm, weight in kg, time since diagnosis in years and DLCO (diffuse capacity of the lung for carbon monoxide), FEV_1 (forced expiratory volume in 1 s) and FVC (forced vital capacity) in % of the predicted value based on age and gender. Data are expressed as range (mean ± SEM).