Antimicrobial Agents and Chemotherapy

Quercetin Induces Apoptosis of *Trypanosoma brucei gambiense* and Decreases the Proinflammatory Response of Human Macrophages

Maria Mamani-Matsuda, Jérôme Rambert, Denis Malvy, Hélène Lejoly-Boisseau, Sylvie Daulouède, Denis Thiolat, Sara Coves, Pierrette Courtois, Philippe Vincendeau and M. Djavad Mossalayi

Antimicrob. Agents Chemother. 2004, 48(3):924. DOI: 10.1128/AAC.48.3.924-929.2004.

Updated information and services can be found at:
http://aac.asm.org/content/48/3/924

These include:

REFERENCES This article cites 36 articles, 11 of which can be accessed free at: http://aac.asm.org/content/48/3/924#ref-list-1

CONTENT ALERTS Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

Journals.ASM.org

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2004, p. 924–929 Vol. 48, No. 3
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.3.924–929.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

Quercetin Induces Apoptosis of *Trypanosoma brucei gambiense* and Decreases the Proinflammatory Response of Human Macrophages

Maria Mamani-Matsuda,[1]* Jérôme Rambert,[1] Denis Malvy,[1,2] Hélène Lejoly-Boisseau,[1] Sylvie Daulouède,[1] Denis Thiolat,[1] Sara Coves,[3] Pierrette Courtois,[1] Philippe Vincendeau,[1] and M. Djavad Mossalayi[1]

Laboratoires d'Immunologie et de Parasitologie, E.A. 3677, Bases Thérapeutiques Anti-Infectieuses et Anti-Inflammatoires, Université Bordeaux II,[1] and Service de Médecine Interne et des Maladies Tropicales, Hôpital Saint André,[2] Bordeaux, and Unilever France, Rueil-Malmaison,[3] France

Received 11 July 2003/Returned for modification 13 October 2003/Accepted 29 November 2003

In addition to parasite spread, the severity of disease observed in cases of human African trypanosomiasis (HAT), or sleeping sickness, is associated with increased levels of inflammatory mediators, including tumor necrosis factor (TNF)-α and nitric oxide derivatives. In the present study, quercetin (3,3′,4′,5,7-pentahydroxyflavone), a potent immunomodulating flavonoid, was shown to directly induce the death of *Trypanosoma brucei gambiense*, the causative agent of HAT, without affecting normal human cell viability. Quercetin directly promoted *T. b. gambiense* death by apoptosis as shown by Annexin V binding. In addition to microbicidal activity, quercetin induced dose-dependent decreases in the levels of TNF-α and nitric oxide produced by activated human macrophages. These results highlight the potential use of quercetin as an antimicrobial and anti-inflammatory agent for the treatment of African trypanomiasis.

Human African trypanosomiasis (HAT), or sleeping sickness, is provoked by the inoculation of *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense* into humans by a tse-tse fly. HAT is considered a reemerging disease, mainly due to the deterioration of health facilities in endemic areas. Blood monocytes and tissue macrophages play a key role in the control of parasite numbers, and an increased number of activated hematopoietic cells are observed during trypanosomiasis (33). Trypanosome-derived products were also shown to activate the generation by macrophages of various proinflammatory mediators including tumor necrosis factor alpha (TNF-α), nitric oxide (NO), and interleukin-1 (IL-1) (7, 19, 28). TNF-α and NO fulfill important functions in host-parasite interactions as they control infections by various pathogens, including *T. b. gambiense* (6, 21, 32). In addition, chronic secretion of macrophage-derived mediators is in part responsible for the pathogenic aspects of HAT (18, 20, 25, 27). Accordingly, a correlation can be made between high levels in serum of TNF-α and disease severity in HAT (25), and successful treatment with melarsoprol significantly reduced the circulating concentration of this cytokine in HAT patients (27).

Only a few drugs are available to treat HAT, and some of them are effective only during the first phase of disease or are difficult to administer because of their high toxicity. These facts led us to investigate the effects of nutrition-derived flavonoids on in vitro interactions between *T. b. gambiense* and human leukocytes. Flavonoids are produced in plants in response to environmental stress such as adverse weather or attacks by insects, animals, or pathogens (37). For humans, the main flavonoid dietary sources are fruits, beverages, vegetables, dry legumes, and cereals. Recently, various purified polyphenolic compounds were defined as strong free-radical scavenging agents that display antitumoral, antimicrobial, and anti-inflammatory activities (3).

Quercetin and its derivatives are among the most common polyphenolic flavonoids present in plants such as onions, ginkgo biloba, and tea and can be absorbed by humans. The anti-inflammatory and antimicrobial activities of quercetin were recently observed in vitro as well as in experimental models (11). This molecule has clearly been shown as a specific inhibitor of Cox-2 and inducible NO synthases (NOS-II) (1). In this study we have investigated the effects of quercetin on the survival of *T. b. gambiense* and on the inflammatory responses of human macrophages.

MATERIALS AND METHODS

Reagents. The following reagents were used in the present work: recombinant human IL-4 (a gift from Schering Plough, Dardilly, France), gamma interferon (a gift from Institut Beaufour, Paris, France), N(6)-(1-iminoethyl)-L-lysine–2HCl and N^G-monomethyl-L-arginine (Sigma-Aldrich, St. Louis, Mo.), and fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies (MAb) to CD23 and CD14 (Immunotech, Marseille Luminy, France). Anti-CD23 MAb (clone 135; a kind gift from Novartis, Basel, Switzerland) was used for macrophage activation while anti-TNF-α MAb (Genzyme, Paris, France) was used for neutralizing experiments. Quercetin (3,3′,4′,5,7-pentahydroxyflavone; >98% purity), resveratrol (*trans*-3,4′,5-trihydroxystilbene), and catechin (*trans*-3,3′,4′,5,7-pentahydroxylflavane) were purchased from Sigma-Aldrich. Quercetin was solubilized in dimethyl sulfoxide while resveratrol and catechin were dissolved in methanol at a concentration of 100 mM. Neither cell nor parasite toxicity (<1/1,000) was observed with working dilutions of the above solvents alone.

Parasites. *T. b. gambiense* (FéoITMAP/1893) and *T. b. gambiense* (OK/ITMAP/1841) were used. These parasites were adapted and maintained in vivo by inoculating normal mice as previously described (32). Parasites were purified from blood by chromatography on DEAE cellulose. Parasite-soluble factors (PSF) were prepared as described in detail elsewhere (2, 7). PSF concentration was determined by a Bradford assay, adjusted to a concentration of 3 mg/ml in phosphate-buffered saline (PBS; pH 8.1) supplemented with 1% glucose, and stored in aliquots at −80°C.

* Corresponding author. Mailing address: Laboratoire d'Immunologie et de Parasitologie, Faculté de Pharmacie, Université Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux cedex, France. Phone: 33 5 57 57 12 43. Fax: 33 5 57 57 12 10. E-mail: maria.mamani@u-bordeaux2.fr.

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

Human leukocytes. Human normal blood samples were obtained from healthy volunteers and tested for the absence of human immunodeficiency virus or hepatitis virus infections. Peripheral blood-derived mononuclear cells (PBL) were obtained by Ficoll gradient separation. Erythrocyte lysis was performed by adding 150 mM NH_4Cl, followed by two washings with Hanks' balanced salt solution. Whole leukocytes were then suspended in McCoy 5A modified culture medium (Sigma-Aldrich) supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, 25 mM HEPES, 0.1 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.2 mM L-cysteine, 5 μg of polymyxin B per ml, and 10% fetal calf serum (FCS) (all from Gibco-Europe, Paisley, Scotland). The culture medium, chemicals, and FCS were endotoxin free and tested for the absence of direct activation effects on human monocytes (CD23 expression and TNF-α production were used as activation markers). Monocytes were subsequently separated from lymphocytes by adherence to plastic dishes coated with FCS as previously described (35). Following this procedure, >90% of the cells expressed CD14 antigen and displayed the cytochemical characteristics of monocytes (35). Nonadherent PBL were incubated with 10^{-6} M phytohemagglutinin-P (Sigma-Aldrich) in order to induce lymphocyte proliferation.

Cell activation. Monocyte-derived macrophages were obtained as described above. These cells could then be activated by coculture with purified trypanosomes (10^6/ml) or by the addition of PSF at a concentration of 80 μg/ml (7). Macrophage differentiation and activation may also be obtained through the stimulation of CD23 antigen as described elsewhere (35). Briefly, CD23 expression was first induced in noninfected monocytes/macrophages (2.10^5/ml) following their incubation with human recombinant IL-4 (10 ng/ml) for 48 h at 37°C. After washing, the cells were tested for their CD23 surface expression (>80% were $CD23^+$) and then incubated in the presence of CD23 MAb (20 μg/ml) for 48 to 72 h. Quercetin, resveratrol, or catechin was added to the cells simultaneously with their activation. Cells were then analyzed for their NO content, and cell supernatants were analyzed for the presence of various inflammatory mediators. Results were analyzed and compared by using the Student *t* test for paired data.

Trypanolysis assay. The lysis assay was performed as previously described (21). Briefly, blood-purified parasites (10^6/ml) were suspended in PBS (pH 8.1) supplemented with 1% glucose. Parasite suspension (100 μl/well) and polyphenols (quercetin, resveratrol, or catechin) or medium alone were added to each well. The percentage of lysed parasites was evaluated 5 h later.

Apoptosis assay. Apoptosis of hemopoietic cells and parasites was quantified by the detection of the externalization of phosphatidylserine at the outer leaflet of the plasma membrane. Briefly, cells and parasites (at least 10^5 for each culture condition) were washed in PBS, incubated with both Annexin V-FITC and propidium iodide, as recommended by the manufacturer (Immunotech, Marseille, France), and analyzed by a Beckman Coulter XL flow cytometer and System II program (Margency, France).

TNF-α and NO determination. Twenty-four hours after macrophage activation, supernatants from the cultures were analyzed for TNF-α levels by the use of a specific enzyme-linked immunosorbent assay (Genzyme, Paris, France). Each sample was tested in duplicate. Since the stable end product of NO, NO_2^-, accumulates in culture medium, supernatants were also assayed 72 h after activation for NO_2^- by using the Greiss reaction modified as detailed elsewhere (16). For intracellular NO measurements, cells (>10^5) were incubated according to Havenga et al. (13), with 10 μM DAF-FM-DA (4-amino-5-methylamino-2′,7′-difluorescein diacetate) (Molecular Probes, Leiden, The Netherlands) for 1 h at 37°C in 1 ml of culture medium. Cells were then washed in PBS and incubated in 0.5 ml of culture medium for 30 min at 37°C. DAF-FM fluorescence emission, which is related to the intracellular NO content, was then analyzed by flow cytometry.

RESULTS

Quercetin directly mediates the apoptosis of *T. b. gambiense*. The addition of quercetin to *T. b. gambiense* cultures resulted in a dose-dependent destruction of parasites as revealed by a microscopic examination made at 24 h postincubation (50% inhibitory concentration [IC_{50}], 10 μM) (Fig. 1). Under similar culture conditions, catechin (10^{-4} to 10^{-6} M), another major tea flavonoid, was unable to affect the in vitro survival of *T. b. gambiense* (<10% death). In an attempt to clarify the trypanocidal mechanism of quercetin, we have tested its effect on parasite death through apoptosis by using Annexin V-FITC and propidium iodide labeling (Fig. 2). The data shown in Fig. 2A indicate that 20 μM quercetin promoted a time-dependent apoptosis of *T. b. gambiense*, as shown by the increase of phosphatidylserine externalization without propidium iodide labeling. Apoptosis was initiated as early as 2 h postincubation with quercetin, and complete cell death was obtained from 24 h postincubation. Therefore, as in mammalian cells, phosphatidylserine externalization may be used as a marker of apoptotic cell death in *T. b. gambiense*. The data in Fig. 2B further show that quercetin induced dose-dependent parasite apoptosis at concentrations as low as 0.1 μM. Combining apoptosis data with cell viability suggests that parasites are highly sensitive to quercetin-mediated toxicity (IC_{50} < 2 μM).

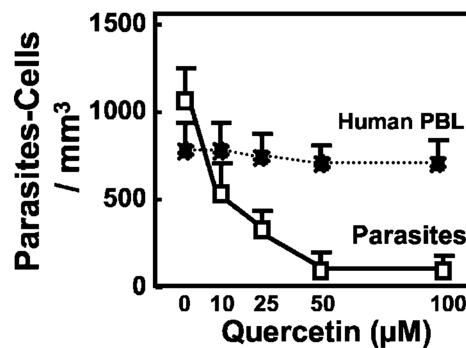

FIG. 1. Quercetin induces *T. b. gambiense* death. Quercetin was added to trypanosomes (10^6/ml) and the number of viable parasites was obtained by a microscopic examination following a 24-h incubation. At similar concentrations, quercetin was not toxic for mitogen (phytohemagglutinin)-activated human PBL (following a 24 h incubation). Results are the means ± standard deviations from five different experiments, each done in duplicate.

The effect of quercetin on normal hemopoietic cells. Recently, we have clearly shown that some flavonoids were safe for nonactivated human leukocytes but had high cytotoxic effects on activated lymphocytes (10). As shown in Fig. 1, quercetin failed to significantly reduce the number of human PBL. In order to assay the hematotoxicity of this molecule, we have incubated purified PBL as well as their monocyte/macrophage subsets with increasing doses of quercetin, with or without *T. b. gambiense*-derived PSF. As shown in Fig. 3, no significant cell apoptosis was observed in hemopoietic cells, while parasites showed high sensitivity to the addition of quercetin. Quercetin toxicity to normal resting or PSF-activated hemopoietic cells was observed only at higher levels than that required for parasite elimination (IC_{50} > 100 μM).

Quercetin decreases TNF-α production by activated human macrophages. A clear relationship exists between the severity of African trypanosomiasis and serum TNF-α levels (25, 27). These observations led us to assess the effects of quercetin on in vitro inflammatory responses of human macrophages to *T. b. gambiense* parasites. Results (Fig. 4) indicate that quercetin significantly (P < 0.0002) inhibited *T. b. gambiense*-mediated activation of TNF-α production. As quercetin had a direct proapoptotic effect on *T. b. gambiense*, we then asked if a decrease in levels of TNF-α could be related to parasite killing and to a decrease in macrophage activation by trypanosomes. Macrophages were thus activated in the absence of *T. b. gambiense*, through CD23 activation antigen (35), and our data

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

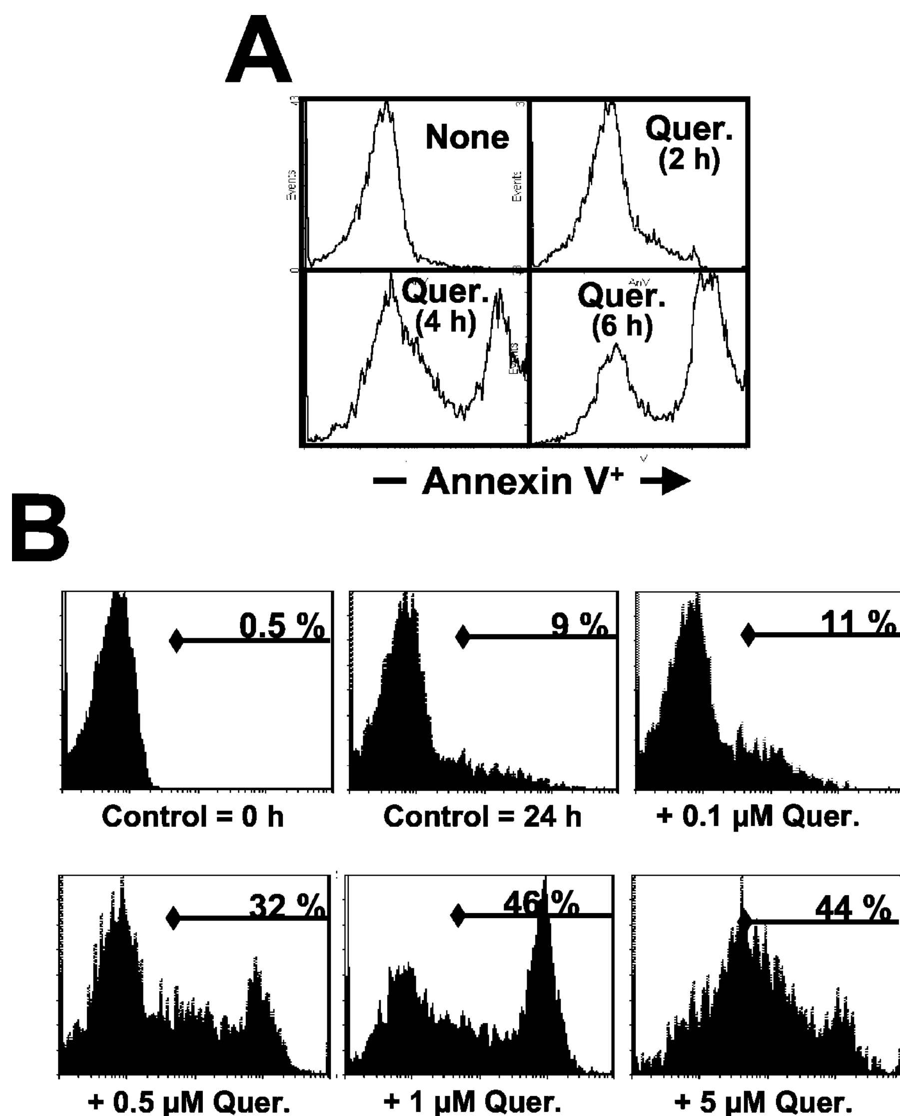

FIG. 2. Quercetin induces *T. b. gambiense* apoptosis. Parasites were incubated in the presence of quercetin, and the phosphatidylserine externalization was analyzed by flow cytometry by using FITC-conjugated Annexin V. (A) Quercetin (20 μM) promoted a time-dependent apoptosis of *T. b. gambiense*, as shown by the binding of Annexin V-FITC. Apoptosis was observed as early as 2 h post incubation. (B) Dose-dependent apoptosis of *T. b. gambiense* by quercetin is observed in 24-h cultures. Significant apoptosis was observed with 0.5 μM quercetin. The results are from one representative experiment out of three. Quer, quercetin.

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

indicate that as in *T. b. gambiense*-activated cells, quercetin significantly ($P < 0.0002$) decreased the levels of TNF-α.

Effect of quercetin on NO production by human macrophages. In addition to TNF-α, macrophages generate NO to eliminate parasites. Chronic exposure to NO may also contribute to the pathophysiology of HAT (6). We therefore assessed the ability of quercetin to modulate NO generation from activated human macrophages. This effect was compared to that of other nutrition-derived polyphenolic compounds, resveratrol and catechin (12), that have been recently reported to modulate NO generation from murine macrophages (5, 24). The addition of quercetin or resveratrol during macrophage activation through the CD23 pathway resulted in a significant inhibition of NO generation from these cells, as detected through the concentration of nitrites in culture supernatants after 72 h of accumulation (Fig. 5A). Such an effect was not observed with cells incubated with catechin-treated cultures (<5% inhibition; range, + 5 to −4%). In addition to nitrite levels, quercetin decreased the levels of intracellular NO in activated macrophages, as detected in a 24-h culture by a fluorescent NO indicator, DAF-FM (Fig. 5B). Thus, quercetin is a potent inhibitor of two major proinflammatory mediators of human macrophages, TNF-α and NO.

DISCUSSION

Our results present the first evidence that quercetin, a food-derived flavonoid, directly promoted the death by apoptosis of *T. b. gambiense*, the causative agent of sleeping sickness. In addition, quercetin was able to inhibit the release of proin-

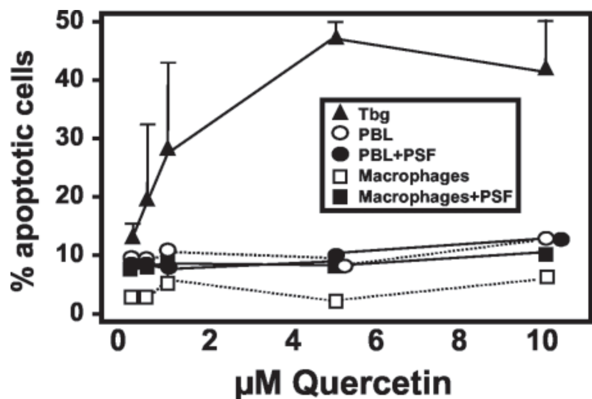

FIG. 3. Quercetin is not toxic for human normal leukocytes. In contrast to *T. b. gambiense* (10^6/ml), quercetin did not induce apoptosis of PBL or human differentiated macrophages activated or not with 80 µg of PSF per ml and cultured in the presence of different doses of quercetin. After incubation for 24 h, phosphatidylserine externalization and DNA content were analyzed by flow cytometry by using Annexin V-FITC and propidium iodide labeling. Results shown are the mean percentages of apoptotic cells or parasites in three isolated experiments.

flammatory mediators, namely TNF-α and NO derivatives, from human activated macrophages.

Quercetin has already been shown to suppress *Toxoplasma gondii* bradyzoite development in vitro through its ability to inhibit the synthesis of Hsp90, Hsp70, and Hsp27, factors that protect virulent parasites from the effects of host immune responses (9). The trypanocidal activity of quercetin demonstrated in the present study correlates with early studies show-

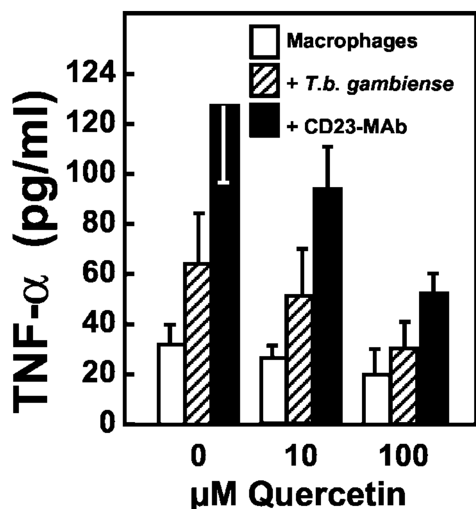

FIG. 4. Quercetin inhibits TNF-α production by activated human macrophages. Human monocyte-derived macrophages (2.10^5/ml) were incubated for 48 h at 37°C with recombinant human IL-4 (10 ng/ml), washed, and reincubated in the presence of CD23 MAb (20 µg/ml). Macrophages were also activated through their cocultures with *T. b. gambiense*. Quercetin was added to macrophages alone or simultaneously with parasites or with CD23 MAb. Cell supernatants were harvested 24 h later, and TNF-α content was quantified. Results are the means ± standard deviations from four distinct donors; each experiment was done in duplicate.

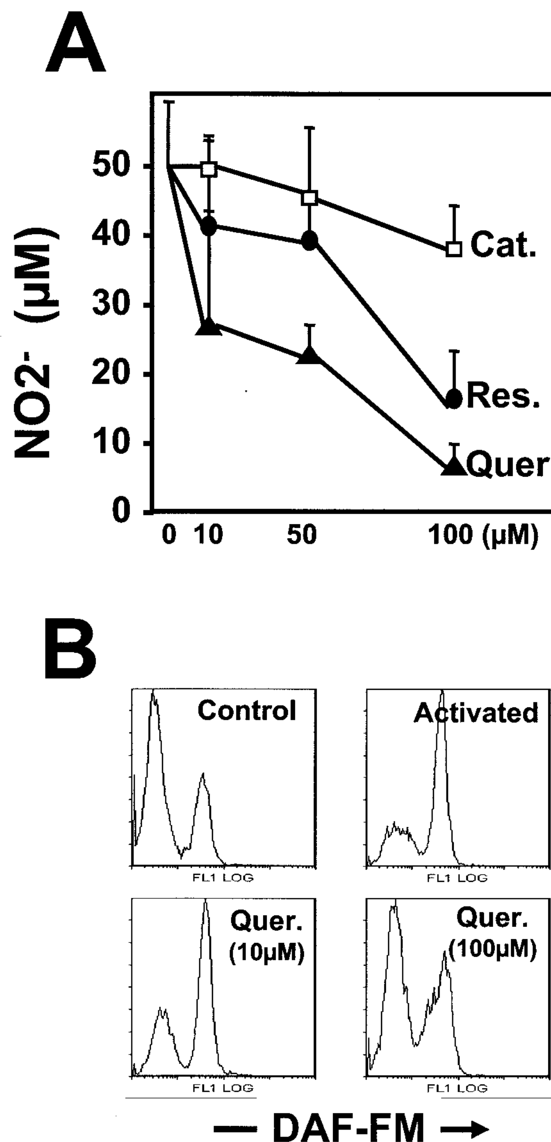

FIG. 5. Quercetin decreases NO generation by human macrophages. (A) Human $CD23^+$ macrophages (2×10^5 cells/ml) were obtained following incubation with IL-4 (10 ng/ml) and were activated by CD23 MAb (20 µg/ml) in the presence or the absence of various dilutions of quercetin, catechin, or resveratrol. Culture supernatants were collected 3 days later, and nitrite levels were measured by Greiss reaction. Nonactivated cells or cells activated in the presence of N(6)-(1-iminoethyl)-L-lysine–2HCl (200 µM; a specific inhibitor of NOS-II activity) had 2 to 7.5 µM NO_2^- levels in their supernatants. Each result is the mean ± the standard deviation from three experiments. (B) Macrophages were incubated in medium alone or in the presence of CD23 MAb and quercetin for 48 h. Cells were then harvested, washed, and incubated with 10 µM DAF-FM-DA for 1 h at 37°C. After washing, cells were incubated in culture medium for 30 min at 37°C and analyzed for the levels of intracellular NO through DAF-FM fluorescence emission, measured by flow cytometry. Quer, quercetin; Cat, catechin; Res, resveratrol

ing the tumoricidal and leishmanicidal activities of food-derived flavonoids in vitro (22, 23). Oral treatment with quercetin protected mice against intraperitoneal encephalomyocarditis virus infections by a macrophage-dependent mechanism (31).

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

In addition to trypanocidal effects, quercetin significantly decreased the proinflammatory responses of human macrophages. Normal human macrophages may be activated through various pathways including lipopolysaccharides, CD23 targeting, or cocultures with trypanosomes or their products. The direct induction of TNF-α production from a monocytoid human cell line was obtained following the incubation of the cells with *T. b. gambiense* or *T. b. gambiense*-derived products and in the absence of T lymphocytes and their cytokines (7). Human macrophage-derived TNF-α and NO are essential for microbicidal activity, but the chronic exposure to these mediators was involved in the appearance of clinical symptoms during trypanosome infections (33). High levels of TNF-α in serum are correlated with disease severity in HAT (25). Present work indicates that quercetin decreases the production of TNF-α and NO from activated human macrophages. The mechanism of the anti-inflammatory effects of quercetin in human macrophages remains to be clarified. In murine macrophages, quercetin was shown to inhibit TNF-α transcription by inhibiting the phosphorylation and the activation of Jun N-terminal kinase/stress-activated protein kinase, leading to the suppression of AP-1 (activating protein-1) activation (36). Quercetin could also inhibit TNF-α production at a posttranscriptional level by inhibiting ERK1/ERK2 and p38 mitogen-activated protein kinase activities, which are important in the posttranscriptional regulation of TNF-α mRNA (36).

The antioxidant property of quercetin is further enforced by our results showing its ability to down-regulate the generation of NO by activated human macrophages. The increased level of NO oxidation products may contribute to the pathophysiology of HAT (6) and other disorders of the central nervous system such as bacterial meningitis (17, 34), cerebral systemic lupus erythematosus (4), and amyotrophic lateral sclerosis (29). Furthermore, NOS-II has also been found in macrophages in multiple sclerosis plaques (8) and in the brain lesions of mice chronically infected with *T. b. brucei* (15). Quercetin has already been shown to offer protection from peroxynitrite-induced consumption of endogenous lycopene beta-carotene and alpha-tocopherol (30).

In conclusion, quercetin may be of potential use for HAT therapy as it acts on both sides of the disease through its anti-inflammatory and trypanocide properties. At its active concentrations, quercetin did not show apparent cytotoxic effects on normal human blood cells at its efficient trypanocidal concentrations and was reported to have no genotoxic or clastogenic effects (14). Compared to other flavonoids, quercetin has relatively good bioavailability following dietary supplementation, and it has been detected in plasma and urine at peak concentrations higher than those required for parasite killing (26). Direct use of various quercetin pharmacological preparations in *T. b. gambiense*-infected animals has been initiated to clarify the therapeutic potential of this molecule.

ACKNOWLEDGMENTS

This work was supported in part by grants from le Conseil Régional d'Aquitaine.

We thank B. Dazey from l'Etablissement de Transfusion d'Aquitaine (blood bank) for blood preparations, and M. Labassa G. Haumont, F. Luchesse, and M. Guy for technical help.

REFERENCES

1. **Banerjee, T., A. Van der Vliet, and V. A. Ziboh.** 2002. Downregulation of COX-2 and iNOS by amentoflavone and quercetin in A549 human lung adenocarcinoma cell line. Prostaglandins Leukot. Essent. Fatty Acids **66:** 485–492.
2. **Bate, C. A., J. Taverne, and J. H. Playfair.** 1989. Soluble malarial antigens are toxic and induce the production of tumour necrosis factor in vivo. Immunology **66:**600–605.
3. **Bravo, L.** 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. **56:**317–333.
4. **Brundin, L., E. Svenungsson, E. Morcos, M. Andersson, T. Olsson, I. Lundberg, and N. P. Wiklund.** 1998. Central nervous system nitric oxide formation in cerebral systemic lupus erythematosus. Ann. Neurol. **44:**704–706.
5. **Cho, D. I., N. Y. Koo, W. J. Chung, T. S. Kim, S. Y. Ryu, S. Y. Im, and K. M. Kim.** 2003. Effects of resveratrol-related hydroxystilbenes on the nitric oxide production in macrophage cells: structural requirements and mechanism of action. Life Sci. **71:**2071–2082.
6. **Clark, I. A., and K. A. Rockett.** 1996. Nitric oxide and parasitic disease. Adv. Parasitol. **37:**1–56.
7. **Daulouede, S., B. Bouteille, D. Moynet, P. De Baetselier, P. Courtois, J. L. Lemesre, A. Buguet, R. Cespuglio, and P. Vincendeau.** 2001. Human macrophage tumor necrosis factor (TNF)-α production induced by *Trypanosoma brucei gambiense* and the role of TNF-α in parasite control. J. Infect. Dis. **183:**988–991.
8. **De Groot, C. J., S. R. Ruuls, J. W. Theeuwes, C. D. Dijkstra, and P. Van der Valk.** 1997. Immunocytochemical characterization of the expression of inducible and constitutive isoforms of nitric oxide synthase in demyelinating multiple sclerosis lesions. J. Neuropathol. Exp. Neurol. **56:**10–20.
9. **Dobbin, C. A., N. C. Smith, and A. M. Johnson.** 2002. Heat shock protein 70 is a potential virulence factor in murine toxoplasma infection via immunomodulation of host NF-kappa B and nitric oxide. J. Immunol. **169:**958–965.
10. **Ferry-Dumazet, H., O. Garnier, M. Mamani-Matsuda, J. Vercauteren, F. Belloc, C. Billiard, M. Dupouy, D. Thiolat, J. P. Kolb, G. Marit, J. Reiffers, and M. D. Mossalayi.** 2002. Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells. Carcinogenesis **23:**1327–1333.
11. **Gee, J. M., and I. T. Johnson.** 2001. Polyphenolic compounds: interactions with the gut and implications for human health. Curr. Med. Chem. **8:**1245–1255.
12. **Harborne, J. B., and C. A. Williams.** 2000. Advances in flavonoid research since 1992. Phytochemistry **55:**481–504.
13. **Havenga, M. J., B. van Dam, B. S. Groot, J. M. Grimbergen, D. Valerio, A. Bout, and P. H. Quax.** 2001. Simultaneous detection of NOS-3 protein expression and nitric oxide production using a flow cytometer. Anal. Biochem. **290:**283–291.
14. **Hu, C. C., W. K. Chen, P. H. Liao, W. C. Yu, and Y. J. Lee.** 2001. Synergistic effect of cadmium chloride and acetaldehyde on cytotoxicity and its prevention by quercetin and glycyrrhizin. Mutat. Res. **496:**117–127.
15. **Keita, M., P. Vincendeau, A. Buguet, R. Cespuglio, J. M. Vallat, M. Dumas, and B. Bouteille.** 2000. Inducible nitric oxide synthase and nitrotyrosine in the central nervous system of mice chronically infected with *Trypanosoma brucei brucei*. Exp. Parasitol. **95:**19–27.
16. **Kolb, J. P., N. Paul-Eugene, C. Damais, K. Yamaoka, J. C. Drapier, and B. Dugas.** 1994. Interleukin-4 stimulates cGMP production by IFN-gamma-activated human monocytes. Involvement of the nitric oxide synthase pathway. J. Biol. Chem. **269:**9811–9816.
17. **Kornelisse, R. F., K. Hoekman, J. J. Visser, W. C. Hop, J. G. Huijmans, P. J. van der Straaten, A. J. van der Heijden, R. N. Sukhai, H. J. Neijens, and R. de Groot.** 1996. The role of nitric oxide in bacterial meningitis in children. J. Infect. Dis. **174:**120–126.
18. **Lejon, V., J. Lardon, G. Kenis, L. Pinoges, D. Legros, S. Bisser, X. N'Siesi, E. Bosmans, and P. Buscher.** 2002. Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of Trypanosoma brucei gambiense sleeping sickness patients before and after treatment. Trans. R. Soc. Trop. Med. Hyg. **96:**329–333.
19. **Lucas, R., S. Magez, R. De Leys, L. Fransen, J. P. Scheerlinck, M. Rampelberg, E. Sablon, and P. De Baetselier.** 1994. Mapping the lectin-like activity of tumor necrosis factor. Science **263:**814–817.
20. **MacLean, L., M. Odiit, D. Okitoi, and J. M. Sternberg.** 1999. Plasma nitrate and interferon-gamma in Trypanosoma brucei rhodesiense infections: evidence that nitric oxide production is induced during both early blood-stage and late meningoencephalitic-stage infections. Trans. R. Soc. Trop. Med. Hyg. **93:**169–170.
21. **Magez, S., M. Geuskens, A. Beschin, H. del Favero, H. Verschueren, R. Lucas, E. Pays, and P. De Baetselier.** 1997. Specific uptake of tumor necrosis factor-alpha is involved in growth control of Trypanosoma brucei. J. Cell Biol. **137:**715–727.
22. **Makita, H., T. Tanaka, H. Fujitsuka, N. Tatematsu, K. Satoh, A. Hara, and H. Mori.** 1996. Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin. Cancer Res. **56:**4904–4909.
23. **Mittra, B., A. Saha, A. R. Chowdhury, C. Pal, S. Mandal, S. Mukhopadhyay,**

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV

S. Bandyopadhyay, and H. K. Majumder. 2000. Luteolin, an abundant dietary component is a potent antileishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol. Med. **6:**527–541.
24. **Murakami, A., K. Matsumoto, K. Koshimizu, and H. Ohigashi.** 2003. Effects of selected food factors with chemopreventive properties on combined lipopolysaccharide- and interferon-γ-induced IκB degradation in RAW264.7 macrophages. Cancer Lett. **195:**17–25.
25. **Okomo-Assoumou, M. C., S. Daulouede, J. L. Lemesre, A. N′Zila-Mouanda, and P. Vincendeau.** 1995. Correlation of high serum levels of tumor necrosis factor-alpha with disease severity in human African trypanosomiasis. Am. J. Trop. Med. Hyg. **53:**539–543.
26. **Rechner, A. R., G. Kuhnle, P. Bremner, G. P. Hubbard, K. P. Moore, and C. A. Rice-Evans.** 2002. The metabolic fate of dietary polyphenols in humans. Free Radic. Biol. Med. **33:**220–235.
27. **Rhind, S. G., B. H. Sabiston, P. N. Shek, A. Buguet, G. Muanga, A. Stanghellini, M. Dumas, and M. W. Radomski.** 1997. Effect of melarsoprol treatment on circulating IL-10 and TNF-alpha levels in human African trypanosomiasis. Clin. Immunol. Immunopathol. **83:**185–189.
28. **Tachado, S. D., and L. Schofield.** 1994. Glycosylphosphatidylinositol toxin of *Trypanosoma brucei* regulates IL-1α and TNF-α expression in macrophages by protein tyrosine kinase-mediated signal transduction. Biochem. Biophys. Res. Commun. **205:**984–991.
29. **Taskiran, D., A. Sagduyu, N. Yuceyar, F. Z. Kutay, and S. Pogun.** 2000. Increased cerebrospinal fluid and serum nitrite and nitrate levels in amyotrophic lateral sclerosis. Int. J. Neurosci. **101:**65–72.
30. **Terao, J., S. Yamaguchi, M. Shirai, M. Miyoshi, J. H. Moon, S. Oshima, T. Inakuma, T. Tsushida, and Y. Kato.** 2001. Protection by quercetin and quercetin 3-O-beta-D-glucuronide of peroxynitrite-induced antioxidant consumption in human plasma low-density lipoprotein. Free Radic. Res. **35:** 925–931.
31. **Veckenstedt, A., and R. Pusztai.** 1981. Mechanism of antiviral action of quercetin against cardiovirus infection in mice. Antiviral Res. **1:**249–261.
32. **Vincendeau, P., S. Daulouede, B. Veyret, M. L. Darde, B. Bouteille, and J. L. Lemesre.** 1992. Nitric oxide-mediated cytostatic activity on Trypanosoma brucei gambiense and Trypanosoma brucei brucei. Exp. Parasitol. **75:**353–360.
33. **Vincendeau, P., M. O. Jauberteau-Marchan, S. Daulouede, and Z. Ayed.** 1999. Immunology of African trypanosomiasis, p. 137–156. *In* M. Dumas, B. Bouteille, and A. Buguet (ed.), Progress in human African trypanosomiasis. Springer-Verlag, Berlin, Germany.
34. **Visser, J. J., R. J. Scholten, and K. Hoekman.** 1994. Nitric oxide synthesis in meningococcal meningitis. Ann. Intern. Med. **120:**345–346.
35. **Vouldoukis, I., V. Riveros-Moreno, B. Dugas, F. Ouaaz, P. Becherel, P. Debre, S. Moncada, and M. D. Mossalayi.** 1995. The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the Fc epsilon RII/CD23 surface antigen. Proc. Natl. Acad. Sci. USA **92:**7804–7808.
36. **Wadsworth, T. L., T. L. McDonald, and D. R. Koop.** 2001. Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor-alpha. Biochem. Pharmacol. **62:**963–974.
37. **Winkel-Shirley, B.** 2002. Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol. **5:**218–223.

Downloaded from http://aac.asm.org/ on February 23, 2013 by PENN STATE UNIV