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Quercetin does not alter lipopolysaccharide-induced fever in rats

Alexandre Kanashiro, Renes R. Machado, David do C. Malvar, Fernando A. Aguiar and Glória E. P. Souza

Abstract

Fever is considered an important component of the acute phase response of the body in defence against invading organisms such as bacteria. Quercetin, an important representative of the flavonoid class, has been extensively studied as an anti-inflammatory agent. In the present study, we investigated the effect of quercetin, administered orally (5, 25 and 50 mg kg^{-1}) or intraperitoneally (50 mg kg^{-1}), on the febrile response induced by either intraperitoneally (50 μg kg^{-1}) or intravenously (5 μg kg^{-1}) injected lipopolysaccharide (LPS from *Escherichia coli*) in rats. In contrast with the well known anti-inflammatory activity of quercetin, the results demonstrate that quercetin, at the doses used, did not alter the fever induced by LPS, regardless of the route of administration.

Introduction

Fever, an important brain-mediated response occurring as part of the acute phase response, is traditionally defined as an elevation in body temperature in response to injury, trauma or invasion by infectious agents (Roth & Souza 2001). Exogenous pyrogens, such as lipopolysaccharide (LPS) isolated from Gram-negative bacteria walls, stimulate host defence cells via interaction with Toll-like receptors, to release several endogenous pyrogens, such as interleukin 1β, interleukin 6, tumour necrosis factor α, macrophage inflammatory protein 1α, RANTES and endothelin 1, which somehow alter the activity of thermoregulatory neurons in the pre-optic area of the anterior hypothalamus (Roth & Souza 2001; Fabricio et al 2005; Melo-Soares et al 2006; Machado et al 2007). This hypothalamic thermoregulatory centre is responsible for the elevation of the corporal temperature via a coordinated series of physiological and behavioural responses, such as peripheral vasoconstriction, immobility and depression, which in themselves facilitate recovery from the precipitating infectious agent (Boulant 2000, 2006; Roth et al 2006).

Despite its adaptive value, the febrile response may be dangerous for patients suffering from autoimmune diseases and coronary atherosclerosis (Moltz 1993). This means that antipyretic therapy may be necessary in patients when the body temperature observed during a febrile response exceeds the physiological benefits. So, the recognition of fever as a response associated with several pathological conditions has increased the scientific interest in the discovery of new compounds with antipyretic properties.

Medicinal plants have long been used in traditional medicine as alternative treatments for a wide range of diseases, including those associated with bacterial infections (Verpoorte 1999; Clardy & Walsh 2004). Recently, several plant-derived secondary metabolites have been described as interfering directly with molecules and/or mechanisms involved in inflammatory processes, inhibiting secondary messengers as well as expressing transcription factors and releasing pro-inflammatory molecules (Haslam 1996; Calixto et al 2003, 2004).

Flavonoids are a large and complex group of polyphenolic compounds widely distributed throughout the plant kingdom (Middleton et al 2000). They are common dietary components of fruits, vegetables and beverages, and are usually present in plant tissue in the

Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Alexandre Kanashiro, Renes R. Machado, David do C. Malvar, Fernando A. Aguiar, Glória E. P. Souza

Correspondence: Glória Emília Petto de Souza, Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo. Avenida do Café s/nº, Campus USP, Ribeirão Preto, SP, Brazil. CEP 14040-903. E-mail: gepsouza@fcfrp.usp.br

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form of glycosides. Quercetin, one of the most widely distributed polyphenolic compounds in the plant kingdom, is the main flavonoid representative of this class. The beneficial effects of this compound on models of inflammation have been investigated in several studies. Quercetin displays a great variety of pharmacological and biological properties and is known to have considerable anti-inflammatory activity, as detected by in-vitro and in-vivo studies (Middleton & Kandaswami 1992; Formica & Regelson 1995). Inhibition of cyclooxygenases, lipoxygenases and xanthine oxidase have been assumed to be a result of quercetin anti-inflammatory properties (Cos et al 1998; Middleton et al 2000). In addition, other studies indicated that quercetin inhibited pro-inflammatory cytokines release (Cho et al 2003) and adhesion molecule expression (Kobuchi et al 1999) as a result of inhibition of transcription factors such as nuclear factor-κB, or p-38 MAP or c-Jun NH_2-terminal kinase pathways.

However, to the best of our knowledge, the antipyretic effect of quercetin has not really been explored in experimental fever models. Therefore, the present study was carried out to evaluate the effect of quercetin on the febrile response after either intraperitoneal or intravenous LPS administration to rats.

Material and Methods

Chemicals

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) and LPS (*Escherichia coli* 0111:B4) were purchased from Sigma Chemical Co. (St Louis, MO, USA), and celecoxib (Celebra) from Pharmacia (São Paulo, Brazil). Cremophor was purchased from BASF (Ludwigshafen, Germany).

Animals and drug administration

Experiments were conducted using male Wistar rats, 180–200 g, housed at 24 ± 1°C under a 12-h light–dark cycle (lights on at 06:00 hours), with free access to food and tap water. The rats were treated orally and intraperitoneally with quercetin at doses of 5, 25 or 50 mg kg^{-1} or with its vehicle alone (saline i.p.; solution of 10%, v/v, cremophor previously dissolved in saline p.o.). At 60 min after quercetin administration, the rats received LPS by either intravenous (5 μg kg^{-1} in 200 μL of sterile saline) or intraperitoneal (50 μg kg^{-1} in 500 μL of sterile saline) injection. For intravenous injection of LPS or saline, the animals were carefully immobilized and the tail was introduced into warm water (≅ 40°C) to promote vasodilatation and facilitate the injection. The tail was then dried, sterilized with cotton soaked with alcohol, and 200 μL of the solutions were injected by using a 1-mL syringe and a stainless steel needle (26 gauge$^{1/2}$). As a positive control, another group of rats received celecoxib (5 mg kg^{-1} p.o.) 30 min before LPS administration. Control animals received an equal volume of vehicle and sterile saline. The study was conducted in compliance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann 1983) and the University of São Paulo Animal Care and Use Committee.

Temperature measurements

The body temperature was manually monitored by gently inserting a small flexible thermistor probe (model 402 coupled to a model 46 telethermometer; Yellow Springs Instruments, Yellow Springs, OH, USA) 4 cm into the rectum, without removing the animals from their home cages. Experimental measurements were conducted at the thermoneutral zone for rats (Gordon 1990) in a temperature-controlled room (28 ± 1°C). On the day of experiment, the baseline temperature was determined 3–4 times at 30-min intervals before any injections until 10:00 hours. Body temperature was continuously recorded at 30-min intervals for 6 h after LPS administration. To minimize core temperature changes due to handling, the animals were habituated to this environment and the procedure twice on the day before the experiment and only those animals displaying mean basal rectal temperatures of between 36.8 and 37.4°C were selected for the study.

Statistical analysis

All variations in rectal temperature were expressed as changes from the mean basal value (ΔT in °C). All results are presented as mean ± s.e.m. and mean baseline temperatures were not statistically different among the groups included in any particular set of experiments. Statistical comparisons were performed by two-way analysis of variance followed by Bonferroni's test using Graph Pad Prism Software (version 3.00 for Windows). Significance was set at $P < 0.05$.

Results

Evaluation of the effect of oral administration of quercetin on LPS-induced fever

In the first set of experiments, we investigated the effect of oral administration of quercetin on fever induced by LPS injected either intravenously or intraperitoneally in rats. For this, the animals were pre-treated with three different oral doses of quercetin (5, 25 or 50 mg kg^{-1}) 1 h before LPS injected either intraperitoneally (50 μg kg^{-1}) or intravenously (5 μg kg^{-1}). At these doses, LPS significantly increased the body temperature of the animals when compared with saline-treated animals (control group). Neither the basal temperature nor the fever induced by either intravenous (Figure 1) or intraperitoneal (Figure 2) LPS injection was affected by the highest dose of oral quercetin (50 mg kg^{-1}). In addition, as expected, the pre-treatment of animals with celecoxib (5 mg kg^{-1}), a selective cyclooxygenase-2 inhibitor, suppressed the LPS-induced fever.

Evaluation of the effect of intraperitoneal administration of quercetin on LPS-induced fever

In order to verify whether the ineffectiveness of quercetin given orally could be related to first passage metabolism or its degradation in the digestive system (Murota & Terao 2003), we investigated the effect of intraperitoneal administration of quercetin on the fever induced by LPS. To achieve this, the

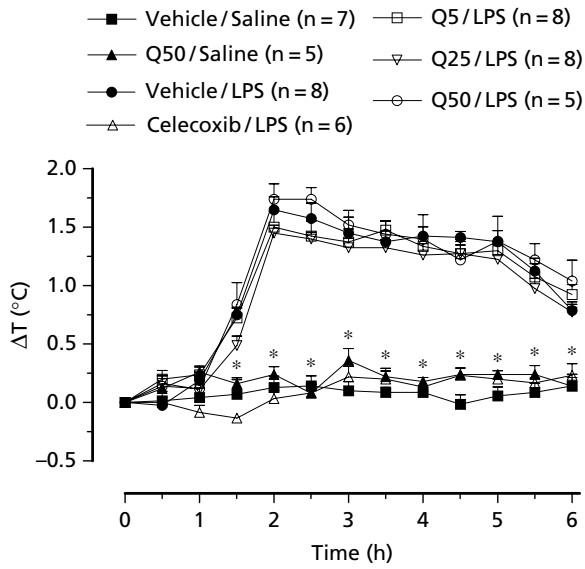

Figure 1 Evaluation of the effect of quercetin (Q) on fever induced by intravenous administration of lipopolysaccharide (LPS) in rats. Quercetin 5, 25 or 50 mg kg^{-1} (p.o.) and celecoxib (5 mg kg^{-1} p.o.) were administered 60 and 30 min before LPS (5 μg kg^{-1} i.v.; time 0 h), respectively. Control animals received the corresponding vehicle and sterile saline (1 mL kg^{-1} i.v.). Values represent the mean ± s.e.m. of variation in rectal temperature (ΔT, °C). Basal temperatures (mean ± s.e.m., °C) were as follows: ■, 36.99 ± 0.06; ▲, 36.94 ± 0.07; ●, 36.93 ± 0.05; □, 36.90 ± 0.05; ∇, 36.90 ± 0.38; ○, 37.00 ± 0.05; Δ, 36.90 ± 0.04. $^*P < 0.05$, significantly different compared with the vehicle/LPS group.

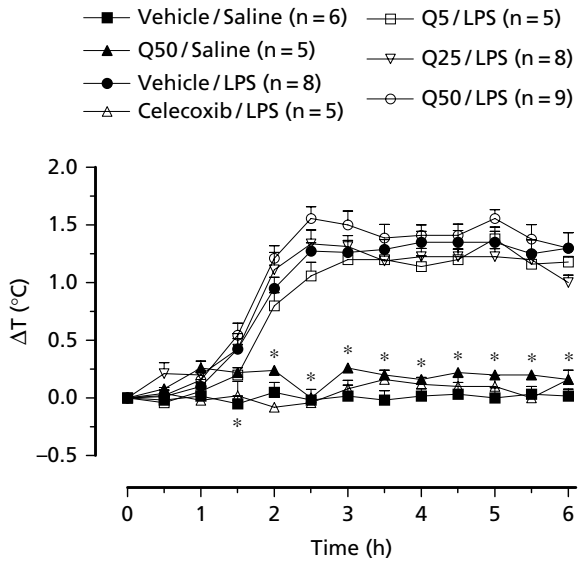

Figure 2 Evaluation of the effect of quercetin (Q) on fever induced by intraperitoneal administration of lipopolysaccharide (LPS) in rats. Quercetin 5, 25 or 50 mg kg^{-1} (p.o.) and celecoxib (5 mg kg^{-1} p.o.) were administered 60 and 30 min before LPS (50 μg kg^{-1} i.p.; time 0 h), respectively. Control animals received the corresponding vehicle and sterile saline (1 mL kg^{-1} i.p.). Values represent the mean ± s.e.m. of variation in rectal temperature (ΔT, °C). Basal temperatures (mean ± s.e.m., °C) were as follows: ■, 37.10 ± 0.07; ▲, 36.96 ± 0.05; ●, 36.93 ± 0.05; □, 36.92 ± 0.07; ∇, 36.95 ± 0.05; ○, 36.97 ± 0.04; Δ, 36.94 ± 0.05. $^*P < 0.05$, significantly different compared with the vehicle/LPS group.

animals were pre-treated with the highest dose of quercetin (50 mg kg^{-1}) 1 h before either intravenous or intraperitoneal administration of LPS (5 and 50 μg kg^{-1}, respectively). Under our experimental conditions, the pre-treatment with quercetin at this dose did not modify the basal temperature or the fever induced by either intravenous (Figure 3) or intraperitoneal (Figure 4) LPS administration.

Discussion

LPS (from the wall of Gram-negative bacteria) is a known potent exogenous pyrogen capable of inducing fever in several animal species (Coelho et al 1992; Zampronio et al 1994; Fabricio et al 1998; Roth et al 2000). Its administration to animals by different routes represents a validated model for studies of neuroimmune interactions, including fever. Fever is a stereotypical response to exogenous pyrogens (such as LPS), which is associated with increased serum and cerebrospinal fluid levels of cytokines, prostaglandin E_2 (PGE_2), pre-formed pyrogenic factor derived from LPS-stimulated macrophages, and endothelin 1 (Zampronio et al 2000; Roth & Souza 2001; Fabricio et al 2005; Melo-Soares et al 2006), and hypothalamic production of PGE_2 (Fitzpatrick & Wynalda 1976). Several different central pathways for fever development have been described: one dependent on prostaglandins, another dependent on endothelin 1

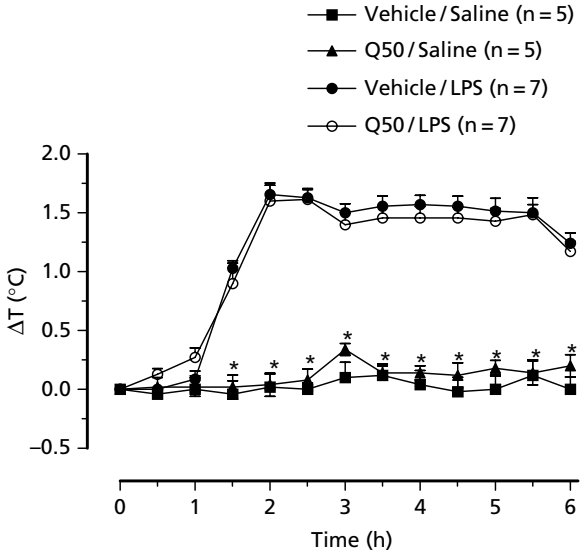

Figure 3 Evaluation of the effect of quercetin (Q) on fever induced by intravenous administration of lipopolysaccharide (LPS) in rats. Quercetin (50 mg kg^{-1} i.p.) was administered 60 min before LPS (5 μg kg^{-1} i.v.; time 0 h). Control animals received the corresponding vehicle and sterile saline (1 mL kg^{-1} i.v.). Values represent the mean ± s.e.m. of variation in rectal temperature (ΔT, °C). Basal temperatures (mean ± s.e.m., °C) were as follows: ■, 36.96 ± 0.11; ▲, 36.90 ± 0.04; ●, 36.87 ± 0.04; ○, 36.93 ± 0.06. $^*P < 0.05$, significantly different compared with the vehicle/LPS group.

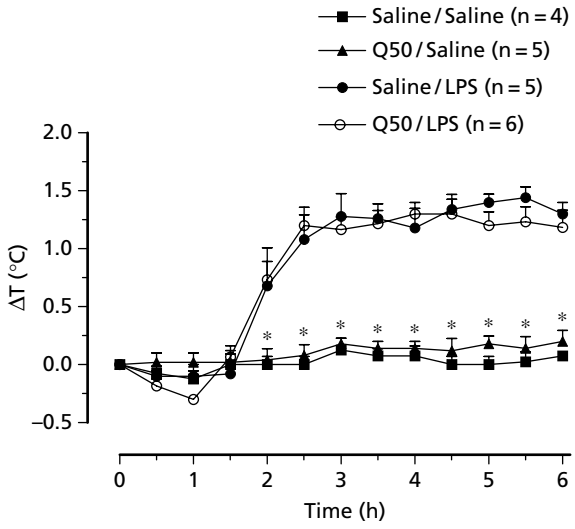

Figure 4 Evaluation of the effect of quercetin (Q) on fever induced by intraperitoneal administration of lipopolysaccharide (LPS) in rats. Quercetin ($50\,mg\,kg^{-1}$ i.p.) was administered 60 min before LPS ($50\,\mu g\,kg^{-1}$, i.p.; time 0 h). Control animals received the corresponding vehicle and sterile saline ($1\,mL\,kg^{-1}$ i.v.). Values represent the mean ± s.e.m. of variation in rectal temperature (ΔT, °C). Basal temperatures (mean ± s.e.m.,°C) were as follows: ■, 36.85 ± 0.06; ▲, 36.92 ± 0.05; ●, 36.98 ± 0.07; ○, 36.95 ± 0.05. $^*P < 0.05$, significantly different compared to the vehicle/LPS group.

and interleukin 1, and another dependent on macrophage inflammatory protein 1α (Fabricio et al 2005; Melo-Soares et al 2006).

The fever produced by intraperitoneal injection of LPS depends not only on the centrally produced mediators (as described above), but also on cytokines produced by peritoneal macrophages such as interleukin 1 and tumour necrosis factor α, as well as on the activation of the sensory afferent vagus nerve (Watkins et al 1995; Werner et al 2003). Several studies have demonstrated that subdiaphragmatic vagotomy prevents the development of LPS-induced fever after intraperitoneal injection of LPS in rats. Moreover, the synthesis of cytokines can be detected in the nodose ganglion soon after the intraperitoneal injection of LPS (Hosoi et al 2005).

Quercetin has been widely studied in herbal medicine as a possible candidate molecule to treat inflammatory chronic diseases such as rheumatoid arthritis and colitis (Comalada et al 2005; Mamani-Matsuda et al 2006), and it also shows anti-inflammatory properties in several animal models of acute inflammation (Sobottka et al 2000; Morikawa et al 2003). However, to our knowledge, there is no evidence of the antipyretic effect of quercetin.

The present study demonstrated that quercetin given intraperitoneally or orally, even at the highest dose ($50\,mg\,kg^{-1}$), did not reduce the fever induced by LPS injected either intraperitoneally or intravenously. It is possible that this compound lacks antipyretic activity because it is ineffective, or has poor effectiveness, in inhibiting PGE_2 production. In fact, Shen et al (2002) demonstrated that quercetin showed anti-inflammatory properties in a PGE_2-independent pathway in an LPS-induced inflammatory model.

Thus, even though quercetin could inhibit cytokine synthesis, other mediators or pathways, for instance those that do not depend on prostaglandin synthesis, such as endothelin 1 and macrophage inflammatory protein 1α, can work in fever development (Fabricio et al 2005; Melo-Soares et al 2006). It is also possible that an ineffective amount of quercetin passively crosses the blood–brain barrier to exert its inhibitory effects on the brain synthesis of these pyrogenic mediators.

Several studies suggest that the immunomodulatory properties of quercetin offer curative effects against inflammatory processes by different mechanisms (Middleton & Kandaswami 1992; Middleton et al 2000). However, this compound has often been studied in in-vitro models, which has led to conflicting reports of its activity. Scientific literature suggests that in-vitro studies with natural compounds could allow for the discovery of new mechanisms of action or targets for the treatment of several diseases including those associated with inflammatory process (Middleton & Kandaswami 1992; Middleton et al 2000). In addition, many studies have come to overstated conclusions such as 'a natural compound with pharmacological properties' from cellular systems that have measured only one aspect of cell function or cell mediator production at one time point. Generally, it is acceptable because studies performed on in-vitro models might approximately reflect in-vivo conditions. However, cellular measures are often non-specific and describe only initial and/or intermediate aspects of the immune response. For example, the febrile response contains a high degree of complexity, so the blockage of one step of this response by a given drug does not clarify whether the cellular event in question would depress/increase such a response.

In fact, in-vitro studies demonstrated that quercetin showed potent antioxidant properties in a different system, which were characterized by free radical production (Cos et al 1998, 2003; Burda & Oleszek 2001), but Boyle et al (2000) observed that rutin, a glycosylated quercetin, did not promote any significant change in the plasma antioxidant status in humans. In addition, Shen et al (2002) demonstrated the inhibitory effects of quercetin on LPS-induced nitric oxide and PGE_2 production in cultured and fresh peritoneal macrophages whereas pre-treatment of rats with quercetin inhibited only nitric oxide production but not the PGE_2 production in a LPS-induced inflammatory response.

One possible explanation for the discrepant effects observed in-vitro and in-vivo could be related to the extensive metabolism of quercetin during the digestive process by enterocytes and enzymes from intestinal flora (before absorption) and by hepatic enzymes (after absorption) (Zhu et al 1994; Murota & Terao 2003). Generally, natural compounds, mainly flavonoids, are highly metabolized in the intestine or liver to glucuronidated, sulfated and *O*-methylated metabolites and/or to monophenolic derivatives. Thus, several researchers suggest that investigative studies on pharmacological properties of natural products in cellular systems should be evaluated with metabolized

compounds present in the circulation and not with those non-metabolized compounds found in nature (Morand et al 1998; Day et al 2000).

Although our results may appear to conflict with previous studies that demonstrated the anti-inflammatory properties of quercetin in different acute models of inflammation (Sobottka et al 2000; Morikawa et al 2003), the present study showed that quercetin, given orally, failed to reduce the fever induced by either intravenous or intraperitoneal injection of LPS. We hypothesized that intraperitoneal injection of quercetin would inhibit LPS-induced fever because this route of administration would protect quercetin from being metabolized by intestinal microorganisms or enzymes from epithelial cells (Blaut et al 2003; Rasmussen & Breinholt 2003). However, it was found that intraperitoneal treatment with quercetin ($50\,mg\,kg^{-1}$) did not reduce fever induced by intravenous or intraperitoneal LPS.

Given the evidence that suppression of fever with antipyretic drugs results in higher morbidity and mortality in infectious diseases (Moltz 1993), our results suggest that quercetin may be useful for the treatment of such conditions since it would not affect this important host defence response.

Conclusion

Although quercetin has been extensively studied as an active pharmacological molecule in many in-vitro and in-vivo inflammation models, we have demonstrated here that quercetin did not show any antipyretic effect in our animal fever model under the investigated conditions (dose, administration route, time protocol of pre-treatment and animal species). The present work suggests that more detailed studies to determine the main metabolites and the bioavailable concentrations after ingestion of natural products such as quercetin need to be performed to provide knowledge about the real activity of compounds isolated from natural sources on human health.

References

Blaut, M., Schoefer, L., Braune, A. (2003) Transformation of flavonoids by intestinal microorganisms. *Int. J. Vitam. Nutr. Res*. **73**: 79–87

Boulant, J. A. (2000) Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin. Infect. Dis*. **5**: 157–161

Boulant, J. A. (2006) Neuronal basis of Hammel's model for set-point thermoregulation. *J. Appl. Physiol*. **100**: 1347–1354

Boyle, S. P., Dobson, V. L., Duthie, S. J., Hinselwood, D. C., Kyle, J. A., Collins, A. R. (2000) Bioavailability and efficiency of rutin as an antioxidant: a human supplementation study. *Eur. J. Clin. Nutr*. **54**: 774–782

Burda, S., Oleszek, W. (2001) Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem*. **49**: 2774–2779

Calixto, J. B., Otuki, M. F., Santos, A. R. S. (2003) Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor-κB (NF-κB) *Planta Med*. **70**: 93–103

Calixto, J. B., Campos, M. M., Otuki, M. F., Santos, A. R. S. (2004) Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med*. **69**: 973–983

Cho, S. Y., Park, S. J., Kwon, M. J., Jeong, T. S., Bok, S. H., Choi, W. Y., Jeong, W. I., Ryu, S. Y., Do, S. H., Lee, C. S., Song, J. C., Jeong, K. S. (2003) Quercetin suppresses proinflammatory cytokines production through MAP kinases and NF-kappaB pathway in lipopolysaccharide-stimulated macrophage. *Mol. Cell. Biochem*. **243**: 153–160

Clardy, J., Walsh, C. (2004) Lessons from natural molecules. *Nature* **432**: 729–737

Coelho, M. M., Souza, G. E., Pelá, I. R. (1992) Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. **263**: R423–R427

Comalada, M., Camuesco, D., Sierra, S., Ballester, I., Xaus, J., Galvez, J., Zarzuelo, A. (2005) In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur. J. Immunol*. **35**: 584–592

Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Poel, B., Pieters, L., Vlietinck, A. J., Vanden Berghe, D. (1998) Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod*. **61**: 71–76

Cos, P., Hermans, N., Calomme, M., Maes, L., De Bruyne, T., Pieters, L., Vlietinck, A. J., Vanden Berghe, D. (2003) Comparative study of eight well-known polyphenolic antioxidants. *J. Pharm. Pharmacol*. **55**: 1291–1297

Day, A. J., Bao, Y., Morgan, M. R. A., Willamson, G. (2000) Conjugation position of quercetin glucoronides and effect on biological activity. *Free Radic. Biol. Med*. **29**: 1234–1243

Fabricio, A. S. C, Silva, C. A. A., Rae, G. A., D'Orléans-Juste, P., Souza, G. E. P. (1998) Essential role of endothelin ET_b receptors in fever induced by LPS (*E. coli)* in rats. *Br. J. Pharmacol*. **124**: 542–548

Fabricio, A. S. C., Rae, G. A., D'Orleans-Juste, P., Souza, G. E. (2005) Endothelin-1 as a central mediator of LPS-induced fever in rats. *Brain Res*. **1066**: 92–100

Fitzpatrick, F. A., Wynalda, M. A. (1976) In vivo suppression of prostaglandin biosynthesis by non-steroidal anti-inflammatory agents. *Prostaglandins* **12**: 1037–1051

Formica, J. V., Regelson, W. (1995) Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol*. **33**: 1061–1080

Gordon, D. J. (1990) Thermal biology of the laboratory rat. *Physiol. Behav*. **47**: 963–991

Haslam, E. (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J. Nat. Prod*. **59**: 205–215

Hosoi, T., Okuma, Y., Matsuda, T., Nomura, Y. (2005) Novel pathway for LPS-induced afferent vagus nerve activation: possible role of nodose ganglion. *Auton. Neurosci*. **120**: 104–107

Kobuchi, H., Roy, S., Sen, C. K., Nguyen, H. G., Packer, L. (1999) Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am. J. Physiol. Cell. Phys*. **277**: 403–411

Mamani-Matsuda, M., Kauss, T., Al-Kharrat, A., Rambert, J., Fawaz, F., Thiolat, D., Moynet, D., Coves, S., Malvy, D., Mossalayi, M. D. (2006) Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators. *Biochem. Pharmacol*. **72**: 1304–1310

Machado, R. R., Soares, D. M., Proudfoot, A. E., Souza, G. E. (2007) CCR1 and CCR5 chemokine receptors are involved in fever induced by LPS (*E. coli*) and RANTES in rats. *Brain Res*. **1161**: 21–31

Melo-Soares, D., Veiga-Souza, F. H., Fabricio, A. S. C., Minano, F. J., Souza, G. E. (2006) CCL3/macrophage inflammatory protein-1 alpha induces fever and increases prostaglandin E2 in cerebrospinal fluid of rats: effect of antipyretic drugs. *Brain Res*. **1109**: 83–92

Middleton, E. Jr, Kandaswami, C. (1992) Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharmacol*. **43**: 1167–1179

Middleton, E. Jr, Kandaswami, C., Theoharides, T. C. (2000) The effects of plants flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol. Rev*. **52**: 673–751

Moltz, H. (1993) Fever: causes and consequences. *Neurosci. Biobehav. Rev*. **17**: 237–269

Morand, C., Crespy, V., Manach, C., Besson, C., Demigne, C., Remesy, C. (1998) Plasma metabolites of quercetin and their antioxidant properties. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. **275**: R212–R219

Morikawa, K., Nonaka, M., Narahara, M., Torii, I., Kawaguchi, K., Yoshikawa, T., Kumazawa, Y., Morikawa, S. (2003) Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. *Life Sci*. **74**: 709–721

Murota, K. N., Terao, J. (2003) Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch. Biochem. Biophys*. **417**: 12–17

Rasmussem, S. E., Breinholt, V. M. (2003) Non-nutritive bioactive food constituents of plants: bioavailability of flavonoids. *Int. J. Vitam. Nutr. Res*. **73**: 101–111

Roth, J., Souza, G. E. (2001) Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz. J. Med. Biol. Res*. **34**: 301–314

Roth, J., Störr, B., Martin, D., Voigt, K., Zeisberger, E. (2000) The role of local induction of tumor necrosis factor by LPS within a subcutaneous air pouch in the development of a febrile response in guinea pigs. *Neuroimmunomodulation* **7**: 169–176

Roth, E., Rummel, C., Barth, S. W., Gerstberger, R., Hubschle, T. (2006) Molecular aspects of fever and hyperthermia. *Neurol. Clin*. **24**: 421–439

Shen, S. C., Lea, W. R., Lin, H. Y., Huang, H. C., Ko, C. H., Yang, L. L., Chen, Y. C. (2002) In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E_2 production. *Eur. J. Pharmacol*. **446**: 187–194

Sobottka, A. M., Werner, W., Blaschke, G., Kiefer, W., Nowe, U., Dannhardt, G., Schapoval, E. E., Schenkel, E. P., Scriba, G. K. (2000) Effect of flavonol derivatives on the carrageenin-induced paw edema in the rat and inhibition of cyclooxygenase-1 and 5-lipoxygenase in vitro. *Arch. Pharm*. **333**: 205–210

Verpoorte, R. (1999) Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. *Drug Discov. Today* **3**: 232–238

Watkins, L. R., Goehler, L. E., Relton, J. K., Tartaglia, N., Silbert, L., Martin, D., Maier S. F. (1995) Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci. Lett*. **183**: 27–31

Werner, M. F., Fraga, D., Melo, M. C., Souza, G. E., Zampronio, A. R. (2003) Importance of the vagus nerve for fever and neutrophil migration induced by intraperitoneal LPS injection. *Inflamm. Res*. **52**: 291–296

Zampronio, A. R., Melo, M. C. C., Silva, C. A. A., Pela, I. R., Hopkins, S. J., Souza, G. E. P. (1994) A pre-formed pyrogenic factor released by lipopolysaccharide stimulated macrophages. *Mediat. Inflamm*. **3**: 365–373

Zampronio, A. R., Hoadley, M. E., Luheshi, G., Rothwell, N. J., de Souza, G. E., Hopkins, S. J. (2000) Interleukin (IL)-6 release and fever induced by a pre-formed pyrogenic factor (PFPF) derived from LPS-stimulated macrophages. *Eur. Cytokine Netw*. **11**: 589–596

Zhu, B. T., Ezell, E. L., Liehr, J. G. (1994) Catechol-*O*-methyltransferase-catalyzed rapid *O*-methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo. *J. Biol. Chem*. **269**: 292–299

Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* **16**: 109–110