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Inhibitory effect of quercetin on carrageenan-induced inflammation in rats

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Abstract

We examined the effect of quercetin on the inflammatory response induced by carrageenan in the rat. Air pouches were induced subcutaneously on the backs of rats and injected with carrageenan. The rats were treated with either vehicle or quercetin at a dose of 10 mg/kg one hour before carrageenan challenge. Fourty-eight hour after carrageenan challenge, the air pourches were removed and analyzed. The volume, protein amounts and cell counts in the exudation obtained from the quercetin-treated animals were significantly reduced compared to those from vehicle-treated animals. The contents of PGE_2, TNF-α, RANTES, MIP-2 and the mRNA for cyclooxygenase-2 were also suppressed in these rats. The histological examination displayed the suppression of the inflammatory response in the pouch tissues from quercetin-treated rats. As the anti-inflammatory effect of the flavonols was more or less at the similar level among the quercetin-, isoquercitrin- or rutin-treated rats, it appeared that the sugar parts did not influence on the anti-inflammatory effect.

Our study indicated that the flavonols modulated the inflammatory response, at least in part, by modulating the prostanoid synthesis as well as cytokine production.
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Keywords: Quercetin; Inflammation; Suppression; Rats; Air pouch formation

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Introduction

Flavonols are the major dietary flavonoid particularly abundant in fruits and vegetables (McCarty, 2001). Quercetin, the main flavonol in the diet, is found not only in these products, but also in drinks such as wine and tea. It is particularly abundant in onions (0.3 mg/g fresh weight) and tea (10–25 mg/L) (Scalbert and Williamson, 2000).

It has been reported that flavonoids possess a number of biological effects such as antiallergic, anti-inflammatory, antiviral, anti-proliferative and anticarcinogenic activities (Scalbert and Williamson, 2000; Parr and Bolwell, 2000). However, most of these biological effects of flavonoids have been demonstrated in the in vitro studies, and are still difficult to evaluate their role in the prevention or treatment of diseases. For example, many aglycones that are effective in the in vitro assay on the tumor growth suppression are not effective in the in vivo assay (Kamei et al., 1996). Quercetin can suppress lipopolysaccharide-induced prostaglandin E_2 production in vitro, but not in vivo (Shen et al., 2002).

Anti-inflammatory effect of quercetin has been shown in several cell culture studies (Pelzer et al., 1998; Ocete et al., 1998; Sato et al., 1997; Shen et al., 2002), but its mechanism of action has not been clear. In this study, we investigated the effect of quercetin on the acute inflammation using the rat air pouch model.

The rat air pouch is a convenient model to study acute inflammation (Sedwick and Lees, 1986). It is formed by the subcutaneous injection of air over several days and is composed of a lining of cells that consists primarily of macrophages and fibroblasts. Injection of carrageenan into the fully formed air pouch produces an inflammatory granulomatous reaction characterized by a production of chemical mediators in the fluid exudates, including prostaglandin and leukotriene, as well as a significant influx of polymorphonuclear leukocytes (PMNs) and macrophages. The degree of non-immunologically-mediated, carrageenan-induced acute inflammation was characterized by the analysis of the contents of the air pouch. This was manifested by the amounts of fluid exudation, protein amounts, numbers of exudate cells and especially neutrophils, as well as the content of the inflammatory mediators. This model has been utilized to estimate the anti-inflammatory effects of the drugs (Masferrer et al., 1994).

In food, flavonoids naturally occur as glycosides, i.e. linked with various sugars. The chemical structures influence on the bioavailability of flavonoid (Manach et al., 1995). Quercetin linked with glucose, i.e. quercetin glucoside (quercitrin), and quercetin linked with rutinose, i.e. quercetin rutinoside (rutin), both occur widely in foods (McCarty, 2001). The sugar moiety also plays an important role in the biological properties. Linkage with glucose, but not other sugars, in the A-ring is determinant for suppressive activity of tumor growth by flavonoids (Kamei et al., 1996). However, there is very little understanding about a possible relationship between flavonoid structure and their anti-inflammatory activity. The influence of sugar attachment on the anti-inflammatory effect was examined using quercetin, rutin and isoquercitrin in this study. The result has shown that these flavonols have similar suppressive effect on the carrageenan-induced acute inflammation. The anti-inflammatory action of the flavonols is partly explained by their capacity to inhibit the production of cytokines and prostaglandin at the inflammatory sites.

Materials and methods

Chemicals

Lambda carrageenan (Type IV), quercetin (3,3′,4′,5′,7-Pentahydroxyflavone) and rutin (quercetin 3β-D-rutinoside: minimum 95% purity) were purchased from Sigma (St. Louis, Mo, USA). Isoquercitrin

(quercetin 3-D-glucoside) was synthesized in our laboratory. These drugs were dissolved in water containing with 1% tween-80 immediately before use.

Animals and diets

Male Wister rats weighing 190 to 200 g were purchased from Clea Japan, (Tokyo, Japan). They were housed, one per cage, in temperature-controlled rooms (23 °C), with a dark period from 6:00 to 18:00 h and with access to food from 6:00 to 18:00 h. They were fed on a standard semipurified diet (CLEA Rodent Diet, CE-2, CLEA Japan) for 2 wk.

Preparation of air pouches

Air pouches were produced by subcutaneus injection of 20 ml of sterile air into intra-scapular area of the back and maintained by re-inflation with 10 ml of air three and six days later as previously mentioned (Morikawa et al., 2003). One ml of 2% sterile carrageenan dissolved in saline was injected directly into the pouch on the next day of the last injection of the air.

Quercetin (10 mg/kg : 2.5–3.0 mg/rat) or vehicle (1% tween-80 in salin) was administered in a 1 ml solution locally one hour before the carrageenan injection when otherwise mentioned. In some experiment, quercetin was administered simultaneous with or one hour after the carrageenan challenge. The animals were sacrificed by inhalation of diethyl ether 48 hr after carrageenan challenge. A small incision was made in the pouch wall, and the contents of the air pouch were carefully removed using sterile Pasteur pipette after injection of 5 ml of ice-cold PBS containing 0.1% EDTA. The total volume of the mixture of exudate was measured and the volume of the exudate was obtained by subtracting 5 ml from the total volume. Protein concentrations of the exudates were measured based on the method of Bradford using Bio-Rad Protein Assay kit (BIO-RAD Laboratories, CA. USA). The protein concentrations multiplied by the volume of exudates made total protein amounts. The exudate cells were separated by centrifugation and the total number of white blood cells was counted and differential counts were performed microscopically after staining air dried smears with Wright stain.

Assay of TNF-α, RANTES, MIP-2 and PGE_2

The activity of tumor necrosis factor-α(TNF-α), RANTES, macrophage inflammatory protein-2 (MIP-2) and prostaglandin E_2 (PGE_2) was determined by enzyme-linked immunosorbent assay (ELISA) with commercially available kits. The content of PGE_2 in the duplicate samples from the exudates was determined with an PGE_2-specific ELISA kit as described by the manufacturer (Amersham Pharmacia Biotech UK Limited, Buckinghamshire, UK). Rat TNF-α, RANTES and MIP-2 activities were determined in the duplicate samples with Cytoscreen™ (BioSource International, Camarillo, Calif.) with detection limits of 15.6, 39 and 10 pg/ml, respectively.

RNA isolation and cDNA synthesis

Total RNA was extracted from the fresh exudate cells using Trizol (GIBCO, Gaitherburg, MD, USA) according to the instructions provided by the manufactures. Five microgram of total RNA was reverse-

transcribed into cDNA using commercially available cDNA synthesis kits (Ready-To-Go You-Prime First-Strand Beads, Amersham Pharmacia Biotech). The tubes were incubated at 30 °C for 10 min, then at 42 °C for 60° min, heated at 95 °C for 5 min and stored at −20 °C until use.

PCR conditions

PCR primers were selected from the published cDNA sequences (Beiche et al., 1996) and commercially synthesized (Amersham Pharmacia Biotech). The following primers were used for the genes of interest: Cox-2: 5′ -forward primer CTGTATCCCGCCCTGCTGGTG and 3′ -reverse primer ACTTGCGTTGATGGTGGCTGTCTT, GAPDH: 5′ -forward primer AGGTCGGTGTCAACGGATTT and 3′ -reverse primer CCTTCCACGATGCCAAAGTT. These primer pairs were found to yield amplified products of the expected sizes of 281 bp for COX-2 cDNA and 504 bp for GAPDH. Aliquots (5 μl) of the reaction were used for PCR amplification with Cox-2- or GAPDH-specific primers using commercially available PCR kits (Ready-To-Go-PCR Beads, Amersham Pharmacia Biotech). Samples were placed in a thermal cycler (PTC-150 MiniCycler Hot Bonnet, MJ Research, INC, MA, USA) and initially denatured at 94 °C for 3 min. They were then denatured at 94 °C for 1 min, annealed at 60 °C (COX-2 primers) or 57 °C (GAPDH primers) for 2 min and extended at 72 °C for 3 min in repetitive cycles. Final extension time was 10 min at 72 °C. Ten microliters of the PCR reaction were resolved by electrophoresis through 1.8% agarose gel. Amplified cDNA bands were detected by ethidium bromide staining and the amounts of mRNA were evaluated by densitometry (Atto Densitograph, Tokyo, Japan) as described previously (Morikawa et al., 2002). The yield of the amplified product was tested to be linear for amount of input RNA and PCR cycle number. For RT-PCR of the samples, the following optimized conditions were chosen: 0.03 μg RNA in 31-35 cycles for assessment of COX-2 and GAPDH expression (data not shown). Expressions were therefore conducted at 0.03 μg RNA/33 cycle for COX-2 and GAPDH mRNA expression.

Histology

The excised pouch tissues were fixed in 10% formalin in 0.01 M phosphate buffer (pH 7.4) and embedded into paraffin wax blocks. Sections were stained with hematoxylin and eosin.

Statistics

The data were expressed as mean ± S.D. Statistical difference was assessed by analysis of Student's t test. Comparison of ANOVA was used to determine significant differences in multiple comparison. Values of $p < 0.05$ were considered significant.

Results

Effect of quercetin on the volume, protein amounts and cell counts in the exudates of the air pouches

The air pouches obtained from 8 rats of either control or the quercetin-treated group (10 mg/kg) were assessed 48 hr after carrageenan challenge. Initially, the inflammatory process in the air pouch was

evaluated by measurement of the volume and protein contents of the exudates, infiltrated exudate cell counts and differential analysis. Significant differences ($p < 0.05$) in the mean volume of the air pouch exudates were observed between controls and quercetin-treated animals (mean: 4.68 vs. 3.30 ml for the rats treated with vehicle vs. quercetin, respectively) as shown in Fig. 1. Total protein amounts and the cell counts in the exudates were also significantly declined in the quercetin-treated animals compared with controls (mean : 19.2 vs. 10.5 mg in the protein amounts, $p < 0.001$; 58.65×10^7 vs. 26.03×10^7 cells in the cell counts, $p < 0.05$, for vehicle- vs. quercetin-treated rats, respectively) (Fig. 1). Differential analysis displayed that more than 80% of the exudate cells obtained from vehicle-treated rats were neutrophils, whereas less than 60% of the exudate cells were neutrophils in the quercetin-treated animals (data not shown).

Effect of quercetin on the release of inflammatory mediators

In the inflammatory sites, numerous cells including monocytes/macrophages, neutrohils, fibroblasts and T lymphocytes generate various inflammatory cytokines. The contents of chemical mediators and cytokines in the exudates from the rats described in Fig. 1 were measured by ELISA. Each sample was measured in duplicate. Data are expressed as mean ± S.D. of the data from 8 rats in each group. Student's t test was used to assess the difference between the quercetin-treated group versus the control group. The concentration of TNF-α, RANTES and PGE_2 in the exudates was markedly decreased than those from vehicle-treated animals (mean: 856 pg/ml vs. 169 pg/ml in TNF-α, $p < 0.01$; 408 pg/ml vs. 259 pg/ml in RANTES, $p < 0.05$; 1055 pg/ml vs. 52 pg/ml in PGE_2, $p < 0.001$ for vehicle- vs. quercetin-treated rats, respectively) (Fig. 2). The concentration of MIP-2 was also reduced, but not statistically significant (mean: 113 pg/ml vs. 98 pg/ml in MIP-2 for vehicle- vs. quercetin-treated rats, respectively) (Fig. 2).

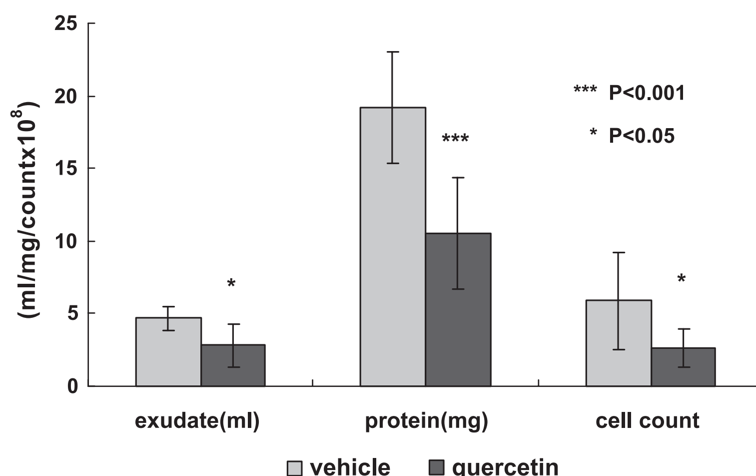

Fig. 1. Effect of quercetin on the volume, protein amounts and cell counts in the exudates of the air pouchs. Rats in groups of 8 rats each were administered quercetin at 10 mg/kg or vehicle in a 1 ml solution, locally, one hour prior to the challenge. Forty-eight hour after carrageenan challenge, the animals were sacrificed and the exudate fluids in the air pouches were collected. The exudate was assessed for WBC counts and the concentration of protein. Data are expressed as mean ± SD of the data and Student's t test was used to assess the difference between the quercetin-treated group versus the vehicle group.

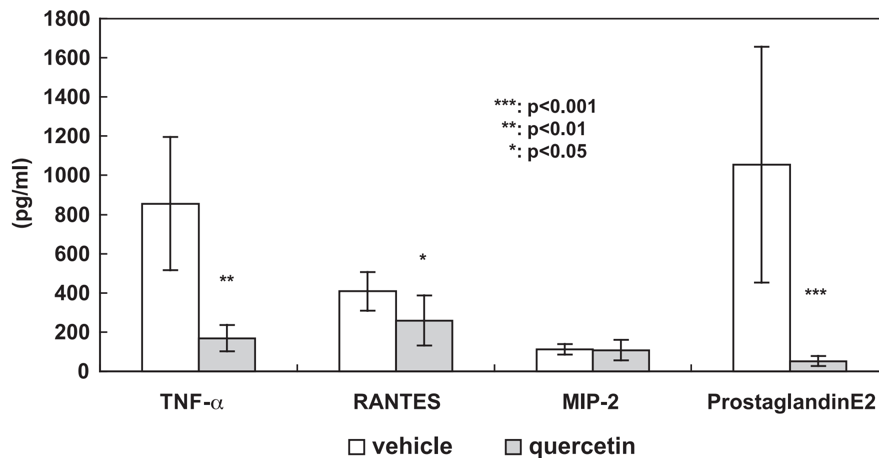

Fig. 2. Effect of quercetin on the release of inflammatory mediators. The contents of TNF-α, RANTES, MIP-2 and PGE_2 in the exudates from the rats described in Fig. 1 were measured by ELISA. Each sample was measured in duplicate. Data are expressed as mean ± SD of the date from 8 rats in each group. Student's t test was used to assess the difference between the quercetin-treated group versus the vehicle group.

Effect of quercetin dosage on the carrageenan-induced acute inflammation

Various doses (0.1, 1, 10 and 100 mg/kg) of quercetin were administered locally one hr before challenging them with 2% carrageenan into 5 groups with 3 rats in each group. All animals were sacrificed at 48 hr after carrageenan administration. Qercetin suppressed the anti-inflammatory response estimated by such as the volume, protein amounts and cell counts in the exudates at concentrations between 10 to 100 mg/kg (Fig. 3).

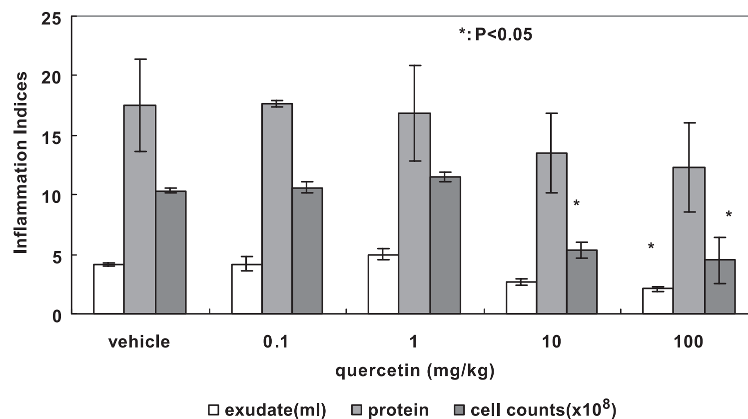

Fig. 3. Dose effects of quercetin on the inflammatory response induced by carrageenan challenge. Various doses of quercetin or vehicle were administered locally one hr prior to carrageenan challenge into 5 groups with 3 in each group and the contents of the exudation in the air pouches were examined. Values are mean ± SD of 3 rats in each group.

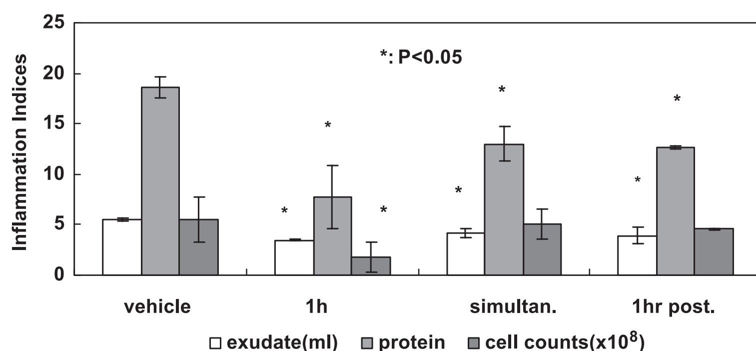

Fig. 4. Timing of quercetin administration for inducing anti-inflammatory response. Quercetin (10 mg/kg) was administered locally at different time points such as one hr before, simultaneous or one hr after into 3 groups with 3 each. In one group with 3 rats, vehicle was administered one hr before the carrageenan challenge. Values are mean ± SD of 3 rats in each group.

Effect of time interval between quercetin pretreatment and carrageenan administration

A 10 mg/kg of quercetin was administered at different time intervals one hr before, simultaneous with, or one hr after the injection of carrageenan into 4 groups with 3 rats each and the exudates were collected when all animals were sacrificed at 48 hr after carrageenan administration. Although the contents of the exudates were reduced in the quercetin-treated rats between the three different time intervals, the most potent effect was induced by the one hr pre-administration (Fig. 4).

Effect of sugar moiety on the anti-inflammatory response

In food, quercetin naturally occurs as the linkage with sugar usually bound at the C-3 position. Rutin and isoquercitrin are the main glycoside forms of quercetin and both occur widely in foods. We

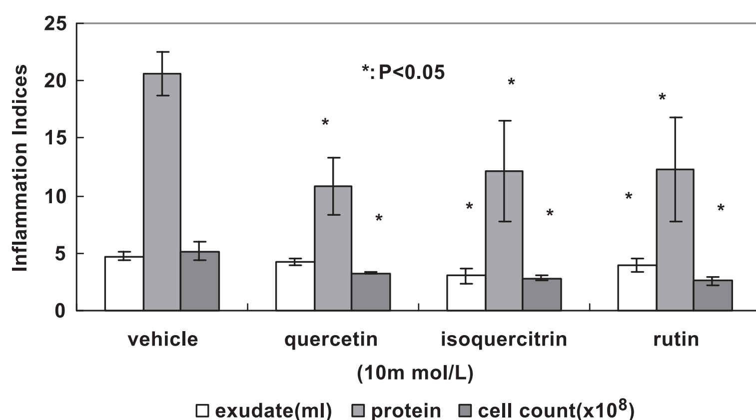

Fig. 5. Effect of sugar moiety on the anti-inflammatory effect. Quercetin dehydrate, isoquercitrin, rutin or vehicle, was administered to 4 groups of 3 rats each, locally, one hr prior to the carrageenan challenge. Forty-eight hour after carrageenan challenge, the animals were sacrificed and the exudation in the air pouches was collected. The exudate was assessed for WBC counts and the concentration of protein. Data are expressed as mean ± SD of the data and ANOVA was used to assess the difference between the compound-treated group versus the vehicle group.

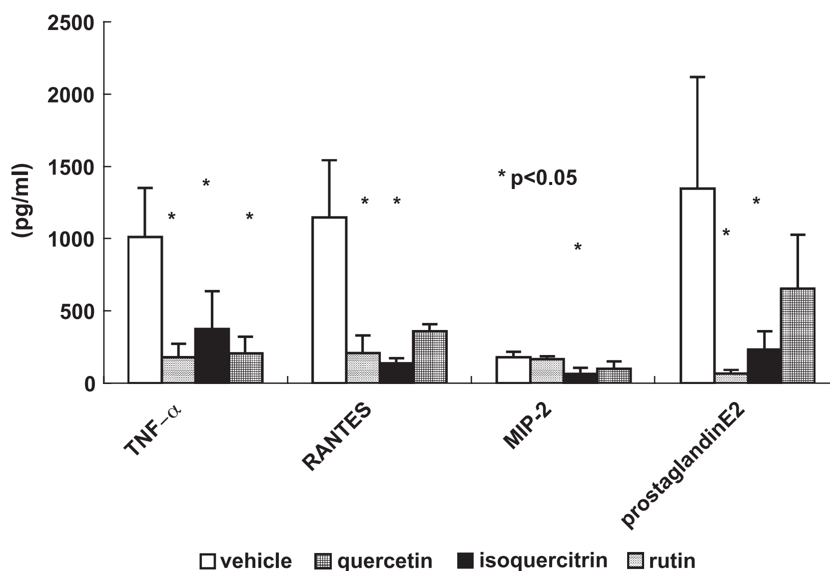

Fig. 6. Effect of the compounds on the release of inflammatory mediators. The contents of TNF-α, MIP-2, RANTES and PGE_2 in the exudation from the rats described in Fig. 6 were measured by ELISA. Each sample was measured in duplicate. Data are expressed as mean ± SD of the data from 3 rats in each group. ANOVA was used to assess the difference between the compound-treated group versus the vehicle group.

examined whether rutin (quercetin rutinoside) and isoquercitrin (quercetin glucoside) were able to suppress the inflammatory response. The compounds were evaluated in the parallel experiments with quercetin for the direct comparison. They showed almost similar level of suppressive activity for the carrageenan-induced inflammation as shown in Fig. 5. The result indicated that anti-inflammatory effect was not affected by the attachment of sugar moiety at the C-3 position.

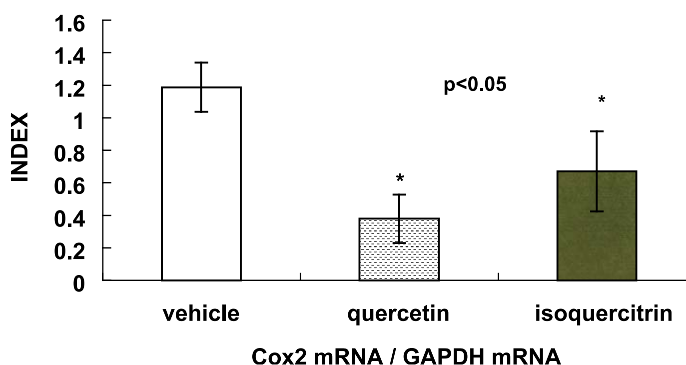

Fig. 7. Effect of quercetin on the expression of COX-2 mRNA in the exudates cells. Total RNA was extracted from the exudates cells of the rats treated with quercetin, isoquercitrin or saline. COX-2 mRNA was examined by RT-PCR as described in materials and methods. The expression of COX-2 was normalized by comparison with the expression of GAPDH. Relative COX-2 mRNA levels were quantified by densitometry and expressed as COX-2/GAPDH optical density ratio. Values are mean ± SD of the data from 3 rats in each group.

Concentrations of TNF-α, RANTES, MIP-2 and PGE_2 in the exudates

The contents of TNF-α, RANTES, MIP-2 and PGE_2 in the exudates from rutin-, isoquercitrin-, quercetin-treated animals were compared with those from vehicle-treated animals. As shown in Fig. 6, the concentrations of TNF-α, RANTES and PGE_2 in the exudates were markedly reduced in the animals treated with the compounds compared with those treated with vehicle ($p < 0.05$). Noteworthy, the suppressive effect of isoquercetin on the MIP-2 release was significant ($P < 0.05$).

Effect of quercetin treatment on COX-2 mRNA expression

It was examined whether or not the decrease of PGE_2 in the flavonol-treated animals was caused by inhibition of up-regulation of COX-2. Expression of COX-2 mRNA within the exudate cells was analyzed by a RT-PCR. Results indicated that COX-2 mRNA expression in the exudate cells from quercetin- or isoquercitrin- treated rats was significantly reduced than those from vehicle-treated rats ($p < 0.05$) (Fig. 7). The results suggested that the flavonols might affect the upregulation of cox-2 mRNA expression in the exudate cells at the inflammatory site.

Histological analysis of inflammatory air pouch

The inflammatory response in the pouch tissues was also examined histologically. The pouch wall is usually consisted of the connective tissue with some adipocytes. The infiltration of many inflammatory cells such as neutrophils, macrophages and lymphocytes, was observed in the pouch wall and the tissue edema contributed to the enlargement of the wall in the vehicle-treated animal (B) (Fig. 8). In contrast, the pouch wall of the quercetin-treated animal was consisted of the thin connective tissue and displayed

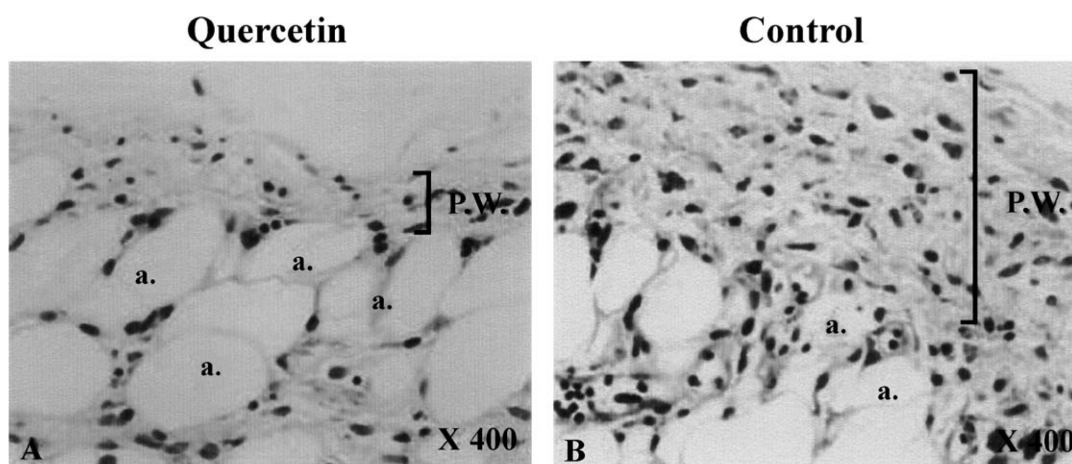

Fig. 8. Histological change in the pouch tissues of quercetin-treated rats. Air pouches were removed from the rats 48 hr after the challenge, fixed with 10% formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin. Inflamed pouch walls (P.W.) from the vehicle-treated rat showed tissue edema with a number of inflammatory cells including neutrophils, macrophages and lymphocytes (original magnification × 400) (B). However, P.W. from quercetin-treated rat showed little tissue edema with few inflammatory cell infiltration (original magnification × 400) (A).

the poor evidence of the acute inflammatory response (A). These histological findings suggested that acute inflammatory response in the pouch wall was suppressed by the quercetin treatment.

Discussion

Here, we have shown that quercetin which was administered one hr before challenge with carrageenan clearly blocked carrageenan-induced inflammation in the rats. This was documented by the decrease of the exudates, protein amounts and inflammatory mediators including TNF-α, RANTES, MIP-2 and PGE_2. Anti-inflammatory effect of quercetin was confirmed in the histological examination. As quercetin was effective when administered simultaneously with or one hr after the carrageenan challenge, quercetin may prevent both early and late phase of the inflammatory response. We also examined whether sugar linkage might influence on the anti-inflammatory effect of quercetin, because the structure of flavonols plays a crucial role on the in vivo tumor suppressive activity (Kamei et al., 1996). In our study, isoquercitrin and rutin displayed similar level of suppressive effect with quercetin, so it appears that the sugar moiety does not act, at least, negatively on the anti-inflammatory effect.

Cytokines produced by inflammatory cells play an important role in the development of inflammatory process. The flavonols, quercetin, isoquercitrin and rutin, suppressed the release of inflammatory mediators including TNF-α, RANTES, MIP-2 and PGE_2 . These results indicated that flavonols inhibited the function of inflammatory cells that produced these inflammatory mediators. It is probable that neutrophils and monocytes are the targets of the anti-inflammatory effects of flavonols, since they contribute toward the majority of exudate cells and are known to produce the inflammatory mediators assessed. It is noteworthy that the absolute number of neutrophils in the exudates was decreased, indicative of the striking anti-inflammatory activity of quercetin. The question is whether a decrease in the number of PMNs is a primary effect of quercetin on neutrophil function or is secondary to the inhibition of the local production of chemotactic or chemokinetic molecules. RANTES, a CC-chemokine, is a chemoattractant for basophils, eosinophils, and T lymphocytes (Baggiolii et al., 1999). MIP-2 is a major CXC-chemokine involved in the migration of PMNs to the sites of inflammation. It is elicited that all PMNs produce large amounts of MIP-2 (Matzer et al., 2001), but rather lesser amounts of TNF-α and PGE_2. Monocytes/ macrophages are the main sources of these chemokines as well as TNF-α and PGE_2 (Baggiolii et al., 1999). Although decrease in the amounts of RANTES and MIP-2 indicates inhibition of the chemokine releases by the flavonols, inhibitory effect for MIP-2 was less potent than those for TNF-α and PGE_2. Therefore, the suppressive effect for the monocytes/ macrophages that produce large amounts of the latter mediators is likely to be a major part of the anti-inflammatory action of quercetin. A recent study has reported that quercetin suppresses TNF-α-mediated stimulation of IL-8 and MCP-1 expression by inhibiting the activation of NF-kB in the cultured human synovial cells (Sato et al., 1997). Thefore, the inhibitory mechanism of quercetin on the cytokine release might depend on the suppression of the gene transcription.

Flavonoids can inhibit 5-lipoxigenase and cyclooxygenase activities in rat peritoneal leukocytes (Laughton et al., 1991) and act as phospholipase A2 inhibitors (Lindahl and Tagesson, 1997). These evidences indicate that flavonols can modulate the prostanoid biosynthetic pathway. Arachdonic acid which is accumulated in the membrane lipid, can be selectively released from the phospholipid pool by chemical or mechanical stimulation, and is subsequently converted to prostaglandins (PGs) by two enzymes, COX-1 and COX-2 (Griffiths, 1999). COX-2 is primarily responsible for PGs produced in inflammation and COX-1 for PGs involved in normal homeostasis. In this regard, COX-2 is up-

regulated in the air pouch and catalyzes the production of large amounts of PGE_2 (Masferrer et al., 1994). The up-regulation of COX-2 associated with the increase of PGE_2 may be major event in carrageenan-induced inflammation. Moreover, non-steroidal anti-inflammatory drugs that inhibit COX-2 isoforms can suppress the inflammation of the rat air pouch (Masferrer et al., 1994). The fact that quercetin suppressed the expression of COX-2 mRNA in the pouch exudates cells indicates that the anti-inflammatory action of quercetin may partly due to suppress up-regulation of COX-2. Actually, quercetin can insert in the membrane lipid bilayers in a concentration- and pH-dependent manner (Movileanu et al., 2000). Alternatively, the decrease of PGE_2 may be induced by the inhibition of the release of TNF-α, because stimulation of macrophages/monocytes, fibroblasts and epithelial cells with cytokines such as IL-1 and TNF-α leads to PGE_2 production (Griffiths, 1999).

Polyphenols are reducing agents and it is commonly referred to as antioxidants that they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others (Scalbert and Williamson, 2000). Quercetin can inhibit increase of IL-8 and MCP-1 gene expression induced by H_2O_2 (Sato et al., 1997). Moreover, quercitrin suppresses inflammatory response in association with antioxidative capacity in the experimental colitis (Sanchez de Medina et al., 2002). These data suggested that the anti-inflammatory effect of quercetin may be due to its antioxidant property. However, other mechanisms should be considered, since it has been indicated that quercetin inhibits activities of several enzymes including protein kinase C (Gschwendt et al., 1983) and tyrosine kinases (Cunningham et al., 1992). Moreover, treatment of hepatoma cells with quercetin induces only a mild oxidative stress despite the suppression of mRNA expression of various antioxidant enzymes (Rohrdanz et al., 2003). Therfore, further study will be required to determine the mechanism of ant-inflammatory effect of quercetin.

The present results were obtained by local injection of the flavonols. We have no data on the anti-inflammatory effect of quercetin by oral administration in this study at present. Bioavailability studies of polyphenols are very limited compared with that available for drugs. The chemical structure of flavonoids determines their rate and extent of intestinal absorption and the nature of the metabolites circulating in the plasma (Scalbert and Williamson, 2000). Previous report describes that bioavailability of quercetin and rutin from the digestive tracts to their plasma transport is less than 5% (Manach et al., 1995). Rutin is absorbed more slowly than quercetin because it is hydrolysed by the cecal microflora, whereas quercetin and quercetin glucoside are absorbed from the small intestine (Manach et al., 1997; Hollman et al., 1999). Absorption of quercetin rutinoside is only twenty percent of quercetin glucoside (Hollman et al., 1999). Other data indicates that absorption of quercetin is enhanced by glycosylation (Hollman et al., 1995). Thus, large uncertainties still remain in the absorption and metabolism of the flavonoids (Scalbert and Williamson, 2000). Therefore, there are many difficulties to deal our subject with oral administration and that is the reason why we have selected the mode of local administration in this experiment. Further study is required to determine the effective oral dosage of the flavonols to suppress inflammatory response.

Anyway, our results have shown that the flavonols including quercetin, isoquercitrin and rutin can suppress inflammatory reaction in vivo. Anti-inflammatory effect of these flavonols suggests the possibility of their therapeutic efficacy in various inflammatory diseases.

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