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# Ajoene, a natural product with non-steroidal anti-inflammatory drug (NSAID)-like properties?

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**Abstract**

The inducible isoform of cyclooxygenase (COX-2) is implicated in the pathogenesis of various inflammatory diseases as well as in carcinogenesis, especially of gastrointestinal tumors. Epidemiological as well as experimental data support a role for constituents of *allium* vegetables, such as garlic and onions, in the prevention of gastrointestinal cancer. Therefore, the aim of the present study was to examine whether the garlic-derived natural product ajoene interferes with the COX-2 pathway by using lipopolysaccharide (LPS)-activated RAW 264.7 cells as *in vitro* model. Ajoene was shown to dose-dependently inhibit the release of LPS (1 $\mu$g/mL)-induced prostaglandin $E_2$ in RAW 264.7 macrophages ($IC_{50}$ value: 2.4 $\mu$M). This effect was found to be due to an inhibition of COX-2 enzyme activity by ajoene ($IC_{50}$ value: 3.4 $\mu$M). Ajoene did not reduce COX-2 expression, but rather increased LPS-induced COX-2 protein and mRNA expression compared to LPS-stimulated cells only. In the absence of LPS, however, ajoene was unable to induce COX-2. The non-steroidal anti-inflammatory drug indomethacin was shown to act similarly in LPS-activated RAW 264.7 cells. These data suggest that ajoene works by a mechanism of action similar to that attributed to non-steroidal anti-inflammatory drugs. This finding may add a novel aspect to the biological profile of the garlic-derived natural product ajoene which might be important for understanding the usefulness of garlic for chemoprevention of gastrointestinal carcinomas. 

*Keywords:* Ajoene; Garlic; Cyclooxygenase 2; RAW 264.7; Macrophage; Lipopolysaccharide

## 1. Introduction

Cyclooxygenases, existing in at least two different isoforms, catalyze the conversion of arachidonic acid to prostaglandins. COX-1 is a constitutively expressed gene, whereas COX-2 is inducible by inflammatory and mitogenic stimuli [1,2]. Although both enzymes carry out essentially the same catalytic reaction [3], pathological effects are attributed mainly to COX-2. Aberrant or excessive expression of COX-2 has been implicated in the pathogenesis of various diseases such as inflammation and arthritis, Alzheimer's disease, and carcinogenesis, especially of gastrointestinal tumors [4–9].

Early reports which pointed to a role of cyclooxygenase in tumorigenesis were observations of elevated levels of prostaglandins in malignant gastrointestinal tissue compared to normal tissue [6,10–12]. Population-based studies detecting a decreased risk of colorectal cancer in persons regularly taking NSAIDs, inhibitors of prostaglandin synthase activity, further supported this notion [13–17]. The findings of dramatically increased COX-2 levels in transformed cells [6–8] and malignant tissue [6,7,9], together with the fact that inhibitors of prostaglandin synthesis protect against carcinogenesis [6,8,13–19], led to the search for COX-2 enzyme inhibitors as potential chemopreventive agents.

Interestingly, epidemiological studies suggest that consumption of garlic (*Allium sativum* L.) and other *allium* vegetables may protect against carcinogenesis. In particular, the development of gastric and colorectal cancers seems to be prevented by *allium* consumption [20–22]. Therefore, we speculated whether *allium* vegetables may contain constituents interfering with the COX-2 pathway.

In this respect, ajoene ((*E*,*Z*)-4,5,9-trithiadodeca-1,6,11-triene-9-oxide), a compound originally isolated from

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*Abbreviations:* COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; and $PGE_2$, prostaglandin $E_2$.

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methanol extracts of garlic [23], seemed to be an interesting candidate: *in vitro* studies demonstrated cytotoxicity against a tumorgenic lymphoid cell line derived from Burkitt's lymphoma [24,25] and the induction of apoptosis in human promyeloleukemic cells [26] by ajoene. In addition, ajoene was demonstrated to influence arachidonate metabolism: in human platelets, ajoene was shown to inhibit the formation of thromboxane $A_2$ and 12-hydroxyeicosatetraenoic acid [27]. COX-1 activity was also found to be inhibited by ajoene using a preparation from sheep seminal microsomes [28]. Furthermore, we recently demonstrated that ajoene *in vitro* is able to inhibit the expression of the inducible nitric oxide synthase [29], an enzyme induced under similar pathophysiological conditions as COX-2 [30,31].

These known *in vitro* effects of ajoene together with the epidemiological background of garlic prompted us to examine whether ajoene interferes with the COX-2 pathway. For this purpose, we used LPS-activated murine RAW 264.7 macrophages as a cell model, since they express high levels of COX-2 [32]. The first aim of our study was to examine whether ajoene alters LPS-induced $PGE_2$ release from RAW 264.7 cells. Second, possible underlying mechanisms leading to changes in $PGE_2$ release after ajoene treatment were examined.

## 2. Materials and methods

### 2.1. Cell culture

RAW 264.7 cells obtained from the American Type Culture Collection (ATCC, TIB 71), were cultured in Dulbecco's modified essential medium with 4 mM L-glutamine and 4.5 g/L of glucose (endotoxin level $<0.005$ endotoxin units/mL, BioWhittaker, Bioproducts), supplemented with 10% heat-inactivated fetal bovine serum (GIBCO BRL Life Technologies). Cells were maintained at 37°, 5% $CO_2$ and used for experiments between passages 5 and 20. Cell stimulation was performed with 1 $\mu$g/mL of LPS (*Escherichia coli*, Serotype 055:B5 Sigma). Ajoene was obtained from Dr. K. G. Wagner (GBF Braunschweig) and dissolved in PBS. NS-398 was purchased from Calbiochem. Acetylsalicylic acid, arachidonic acid, $PGE_2$ and dexamethasone were from Sigma.

### 2.2. $PGE_2$ release

To determine $PGE_2$ accumulation from endogenous arachidonic acid, cells were seeded in 96-well plates ($8 \times 10^4$/200 $\mu$L/well), cultured for two days and, after supernatants were replaced by fresh medium, incubated with or without LPS in the absence or presence of the test compounds for 20 hr. $PGE_2$ was measured in cell culture supernatants of RAW 264.7 macrophages by radioimmunoassay using [$^3$H]$PGE_2$ obtained from Amersham Pharmacia Biotech and polyclonal antiserum to $PGE_2$ (Cayman Chemical) from SPI-bio. Experiments were performed at least three times in triplicate.

### 2.3. COX-2 enzyme activity

Cells were seeded as described above. Measurement of COX-2 enzyme activity was performed according to Nakatsugi *et al*. [33]. Briefly, before activation, cells were treated with acetylsalicylic acid (250 $\mu$M) for 30 min to irreversibly inactivate COX-1. Thereafter, cells were washed with PBS and fed with fresh medium. Induction of COX-2 was achieved by adding LPS for 20 hr. Then, medium was aspirated and cells washed with PBS again and supplied with fresh medium (fetal bovine serum-free). Test compounds were preincubated for 30 min before exogenous arachidonic acid was added. After 15 min, supernatants were removed and $PGE_2$ measured by radioimmunoassay. Experiments were performed at least three times in triplicate.

### 2.4. Cell viability (MTT assay)

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan [34]. After the supernatants were removed for $PGE_2$ determination, cells were incubated at 37° with MTT (0.5 mg/mL) for 45 min. The medium was aspirated and cells were solubilized in dimethyl sulfoxide (250 $\mu$L) for at least 2 h in the dark. The extent of reduction of MTT was quantified by O.D. measurement (550 nm).

### 2.5. Western blot analysis

Macrophages, grown in 6-well plates to confluence, were incubated with or without LPS in the absence or presence of the test compound. Cells were washed with ice-cold PBS and stored at $-70$° until further analysis. Frozen plates were put on ice and cells were lysed in 1% Triton X-100, 0.15 M NaCl, and 10 mM Tris-HCl pH 7.4 with complete™ (Boehringer Mannheim) for 30 min. Lysates were homogenized through a 22 G needle and centrifuged at 10,000 *g* for 10 min at 4°. The supernatants were collected and protein was measured by the method according to Bradford [35]. Cell lysates, containing equal amounts of protein, were boiled in SDS sample buffer for 5 min before running on a 10% SDS–polyacrylamide gel. Proteins were transferred to polyvinylidene fluoride membranes (Immobilon-P™, Millipore). Membranes were blocked with 5% fat-free dry milk in TBS-T pH 8.0 (Tris-buffered saline [50 mM Tris, pH 8.0, 150 mM NaCl] with 0.1% Tween 20) and then incubated with a mouse immunoglobulin $G_1$ against COX-2 (Transduction Laboratories) diluted 1:250 overnight at 4°. After washing 3 times with TBS-T, COX-2 was visualized by an anti-mouse IgG:horseradish peroxidase conjugate and the enhanced chemiluminescence system (ECL™, Amersham

Pharmacia Biotech). Signal intensities were evaluated by densitometric analysis (Kodak Digital Science™ Image Station 440CF, NEN Life Science Products).

### 2.6. RNA extraction and Northern blot analysis

Confluent macrophages (75-cm$^2$ flasks) were incubated with or without LPS in the absence or presence of the test compounds for 4 hr. RNA extraction and Northern blot were performed according to [36]. Briefly, RNA was extracted by the guanidine thiocyanate/cesium chloride method, followed by fractionation on a 1.2% agarose gel (20 $\mu$g total RNA per lane), blotted on a nylon membrane (0.2 $\mu$m, GLW), and cross-linked by UV (UV Stratalinker 1800, Stratagene). The blots were prehybridized (4 hr, 69°, 50% formamide, 50 mM Na-phosphate buffer pH 7, 1 M NaCl, 2.5 × Denhardt's solution, 0.2% SDS, and 10 mM EDTA) and hybridized under the same conditions overnight to a $^{32}$P-labeled murine TIS10/COX-2 cRNA probe (2 × 10$^6$ cpm/mL). The $^{32}$P-labeled TIS10/COX-2 cRNA was obtained using [$^{32}$P]UTP (50 $\mu$Ci), a T7 RNA polymerase transcription system (Stratagene), and an *Eco*RI linearized 2.3-kb TIS10 cDNA subcloned in a pGem7 vector kindly provided by Dr. H. R. Herschman. Blots were washed in 0.1 × sodium chloride–sodium citrate buffer/1% SDS at room temperature for 10 min, followed by a 1-hr wash at 70° and were then exposed to an x-ray film (Hyperfilm MP, Amersham Pharmacia Biotech) using intensifying screens at −70°. Evaluation of signal intensities was performed by densitometric analysis. As a control for mRNA loading, membranes were rehybridized with a $^{32}$P-labeled $\beta$-actin probe (2 × 10$^6$ cpm/mL) using the protocol described above. The same protocol was also used for iNOS hybridization using a $^{32}$P-labeled iNOS cRNA probe as described [29].

### 2.7. Statistical analysis

$PGE_2$ determination and cell viability were performed in triplicate. All experiments were repeated at least twice. Results are expressed as mean values ± SEM. Statistical comparisons were made by ANOVA followed by a Dunnett's multiple comparison test. $P$ values $<0.05$ were considered significant.

## 3. Results

### 3.1. Influence of ajoene on $PGE_2$ release from LPS-activated murine macrophages

Treatment of RAW 264.7 cells with LPS leads to induction of COX-2, which converts LPS-induced endogenous arachidonic acid to $PGE_2$ [32]. RAW 264.7 cells treated with LPS (1 $\mu$g/mL) in the presence of ajoene (1–10 $\mu$M) for 20 hr showed a dose-dependent decrease in $PGE_2$ release as depicted in Fig. 1A. The $IC_{50}$ value was calculated to be 2.4 $\mu$M. After supernatants were removed for $PGE_2$ measurement, cell viability was determined to exclude the possibility that the observed effect was due to cytotoxicity. In all cases, cell viability was >90% (Fig. 1B). In the absence of LPS, ajoene did not affect $PGE_2$ production compared to uninduced cells (control) (Fig. 1A).

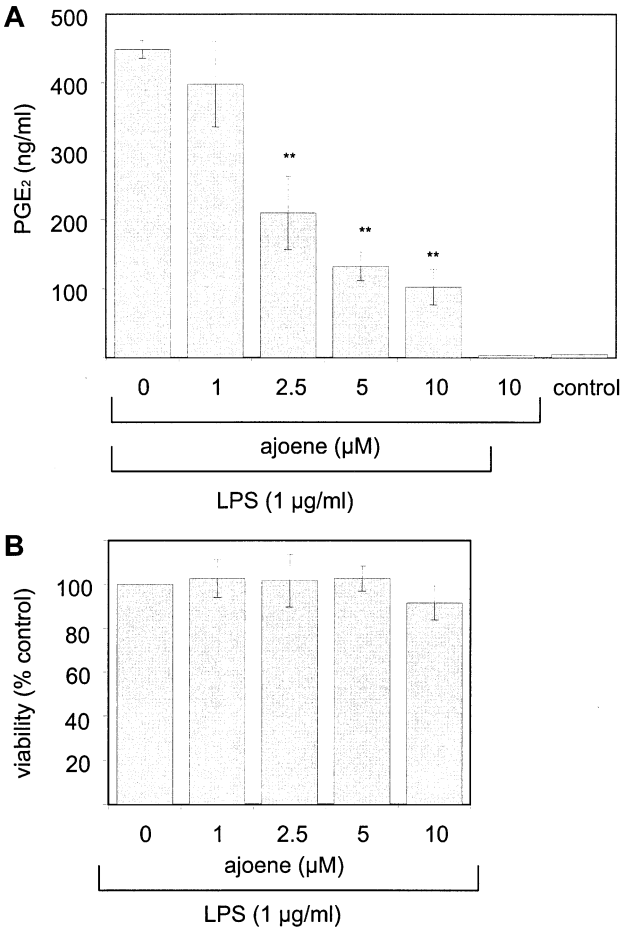


Fig. 1. (A) Influence of ajoene on $PGE_2$ accumulation in cell culture supernatants of LPS-stimulated or unstimulated RAW 264.7 macrophages. RAW 264.7 cells were activated with LPS (1 $\mu$g/mL) in the absence or presence of ajoene (1–10 $\mu$M) for 20 hr, treated with ajoene only (10 $\mu$M), or left untreated (control). $PGE_2$ concentration was determined by radioimmunoassay. Values are given in ng/mL of $PGE_2$. (B) Influence of ajoene on cell viability in the presence of LPS (1 $\mu$g/mL). After cell culture supernatants were removed for $PGE_2$ measurements, cell viability was determined by the MTT assay as described under Materials and Methods. Bars represent mean values ± SEM of at least three independent experiments, each performed in triplicate. ** $P < 0.01$ (ANOVA/Dunnett).

### 3.2. Influence of ajoene on COX-2 mRNA levels in LPS-activated murine macrophages

In an attempt to find the underlying mechanism leading to reduced $PGE_2$ release after ajoene treatment, we first examined the influence of ajoene on LPS-induced COX-2 mRNA levels, since ajoene was recently found to interfere

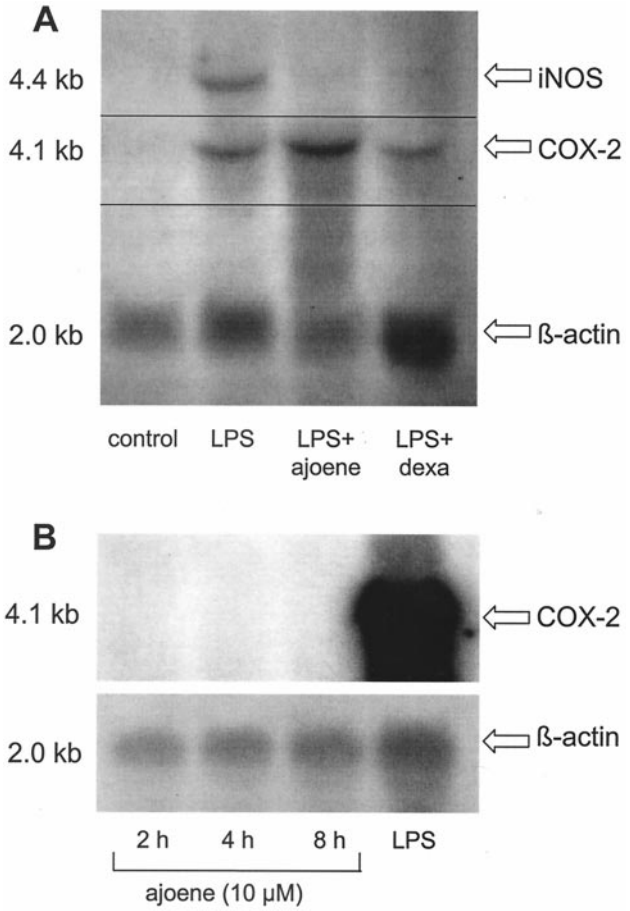


Fig. 2. (A) Representative Northern blot experiment showing the influence of ajoene on LPS-induced COX-2 and iNOS mRNA expression. Total RNA was isolated from macrophages, which were either unstimulated (control), stimulated with LPS (1 $\mu$g/mL), treated with LPS (1 $\mu$g/mL) and ajoene (10 $\mu$M), or treated with LPS (1 $\mu$g/mL) and dexamethasone (dexa, 10 $\mu$M) for 4 hr. Blotted RNA (20 $\mu$g) was hybridized using $^{32}$P-labeled cRNA probes for either iNOS, COX-2, or $\beta$-actin. Similar results were obtained in three independent experiments. (B) Representative Northern blot experiment showing COX-2 mRNA expression in RAW 264.7 cells both after treatment with ajoene (10 $\mu$M, 2–8 hr) in the absence of LPS and after treatment with LPS only. For hybridization, COX-2 and $\beta$-actin probes were used as in (A).

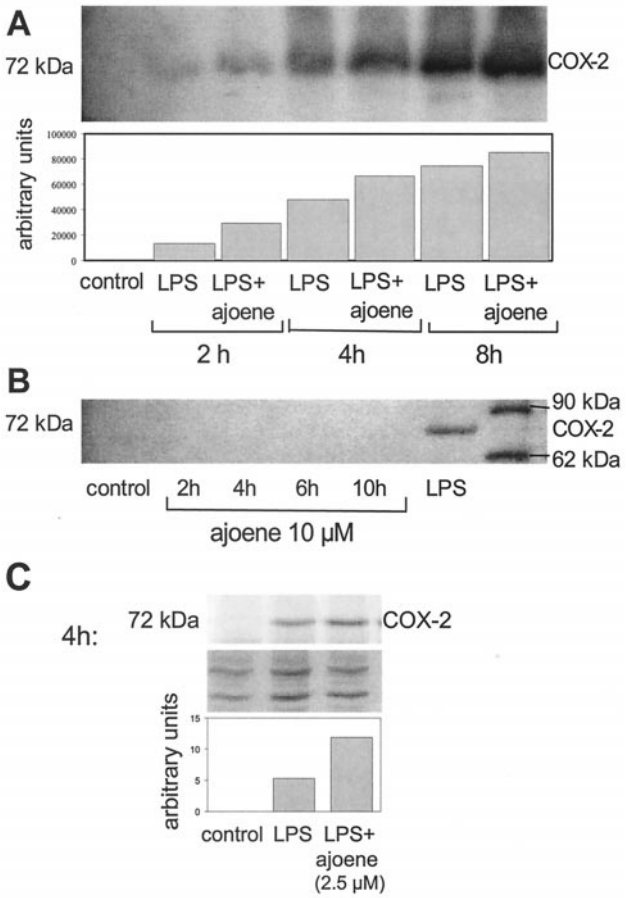


Fig. 3. Representative Western blots. (A) COX-2 protein expression in RAW 264.7 cells stimulated for 2–8 hr with LPS (1 $\mu$g/mL) only, or LPS and ajoene (10 $\mu$M). The densitometric evaluation of the blot is depicted below. Lane designations are identical for the blot and the histogram. (B) COX-2 protein expression in RAW 264.7 cells after treatment with ajoene (10 $\mu$M, 2–10 hr) only, compared to cells treated with LPS (1 $\mu$g/mL, 10 hr) only. (C) COX-2 expression in RAW 264.7 cells stimulated for 4 hr with LPS (1 $\mu$g/mL) only, or LPS and ajoene (2.5 $\mu$M) (upper panel). The densitometric evaluation of the blot, normalized by protein band intensities after Coomassie staining of the gel (middle panel), is depicted in the lower panel. Lane designations are identical for the blot, the gel, and the histogram. Cell lysates were separated on a 10% SDS–PAGE and transferred to polyvinylidene fluoride membranes. Monoclonal mouse antibodies against COX-2 protein were used. Results are representative of three separate experiments.

with the induction of another inducible enzyme, iNOS [29]. We showed in time–course experiments that RAW 264.7 cells expressed COX-2 mRNA half-maximally 4 hr after LPS induction (data not shown), the suitable time point to detect differences in COX-2 mRNA levels in the absence or presence of ajoene. Therefore, Northern blot experiments were performed after 4 hr of incubation. Surprisingly, treatment with LPS in the presence of 10 $\mu$M ajoene, a dose that reduced $PGE_2$ release by about 75–80% (Fig. 1A), led to approx. 1.5- to 2.5-fold enhanced COX-2 mRNA levels compared to levels reached by LPS stimulation only. The representative Northern blot depicted in Fig. 2A shows COX-2 mRNA levels of RAW 264.7 cells treated for 4 hr: 10 $\mu$M ajoene, which under the same conditions almost completely abrogated iNOS mRNA expression, enhanced COX-2 mRNA levels markedly compared to cells stimulated with LPS only. Fig. 2B demonstrates that ajoene in the absence of LPS did not induce COX-2 mRNA.

### *3.3. Effect of ajoene on COX-2 protein levels*

Increased COX-2 mRNA steady-state levels may lead to increased COX-2 protein levels. Therefore, immunoblot analyses were performed to examine the influence of ajoene on LPS-induced COX-2 protein levels. The representative Western blot in Fig. 3A demonstrates that at early time points (2–8 hr), when COX-2 protein levels had not yet reached maximal levels, ajoene showed a co-stimulatory

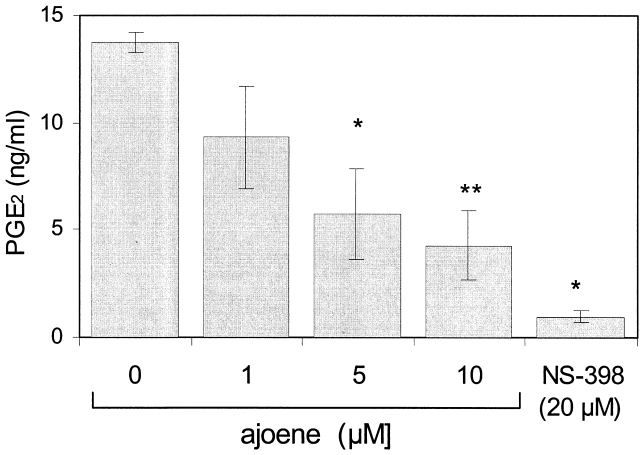


Fig. 4. Reduction of COX-2 enzyme activity in LPS-activated RAW 264.7 macrophages treated with ajoene (1–10 $\mu$M). RAW 264.7 macrophages, in which COX-1 was irreversibly inactivated by acetylsalicylic acid, were activated by LPS (1 $\mu$g/mL) for 20 hr to induce COX-2. After cells were supplied with fresh fetal bovine serum-free medium, they were either left untreated, or treated with ajoene (1–10 $\mu$M) or NS-398 (20 $\mu$M). The reaction was started by adding arachidonic acid. $PGE_2$ was measured by radioimmunoassay. Values are given in ng/mL of $PGE_2$. Bars represent mean values ± SEM of three independent experiments, each performed in triplicate. * $P < 0.05$, ** $P < 0.01$ (ANOVA/Dunnett).

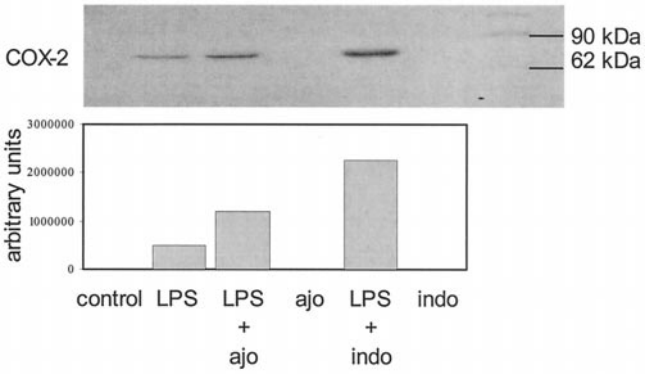


Fig. 5. Increase in LPS-induced COX-2 protein expression after ajoene and indomethacin treatment. The representative Western blot shows the COX-2 protein expression in cells either left untreated or treated with the following: LPS (1 $\mu$g/mL); LPS and ajoene (10 $\mu$M); ajoene (10 $\mu$M) alone; LPS and indomethacin (1 $\mu$M); or indomethacin (1 $\mu$M) alone. The densitometric evaluation of the blot is depicted below. Lane designations are the same for the blot and the histogram. Cell lysates were separated on a 10% SDS–PAGE and transferred to polyvinylidene fluoride membranes. Monoclonal mouse antibodies against COX-2 protein were used. Results are representative of three separate experiments.

effect compared to LPS only. Although the effect was consistently observed, it was much less pronounced than the effects of ajoene on the mRNA level. As can be seen in Fig. 3B, ajoene did not induce COX-2 expression in the absence of LPS. Expression of COX-1 was unaltered compared to control cells after treatment with 10 $\mu$M ajoene for 2–12 hr (data not shown).

To examine whether the observed co-stimulatory effect of ajoene (10 $\mu$M) on COX-2 expression in LPS-activated RAW 264.7 cells also occurs at lower ajoene concentrations (2.5 $\mu$M), when the inhibitory effect of ajoene on $PGE_2$ synthesis is only half-maximal, we performed additional Western blot experiments. The representative Western blot in Fig. 3C shows that after 4 hr of stimulation, ajoene (2.5 $\mu$M) also increased the COX-2 protein level compared to stimulation with LPS only.

### *3.4. Influence of ajoene on COX-2 enzyme activity in LPS-activated intact cells*

Ajoene did not reduce LPS-induced COX-2 mRNA or protein expression. The observed inhibitory effect of ajoene on the $PGE_2$ release in LPS-activated RAW 264.7 cells might therefore be due to a direct inhibition of COX-2 enzyme activity by ajoene. To investigate this possibility, we used intact cells in which COX-2 was induced by LPS and exogenous arachidonic acid as substrate. Since exogenous arachidonic acid can be utilized by COX-1 and COX-2 to produce $PGE_2$ [32,33], COX-1 was irreversibly inactivated by acetylsalicylic acid before COX-2 was induced with LPS. Fig. 4 indeed shows that ajoene was able to inhibit the conversion of exogenous arachidonic acid to $PGE_2$ dose-dependently. The $IC_{50}$ value determined (3.4 $\mu$M) was very similar to that calculated for ajoene-dependent inhibition of $PGE_2$ accumulation in LPS-activated RAW 264.7 cells.

### *3.5. Influence of indomethacin on LPS-induced COX-2 protein expression in murine macrophages*

Inhibition of prostaglandin synthesis by directly interfering with the cyclooxygenase enzyme is a common mechanism of NSAIDs. Since ajoene seemed to act like an NSAID, we were interested as to whether indomethacin, an NSAID that inhibits enzyme activity of COX-1 and COX-2, might affect COX-2 protein expression in LPS-activated RAW 264.7 cells in a similar way as ajoene. A representative Western blot depicted in Fig. 5 shows that indomethacinlike ajoene enhanced COX-2 protein expression, but was unable to induce COX-2 in the absence of LPS. Densitometric evaluations of three blots using tubulin expression as control for applied protein quantities revealed an increase in LPS-induced COX-2 protein after ajoene treatment of approx. 65% and after indomethacin (1 $\mu$M) treatment of about 70%. The selective COX-2 inhibitor NS-398 (20 $\mu$M) showed an increase of about 40% (data not shown).

## 4. Discussion

The present study demonstrates that the natural product ajoene, originally found in methanol extracts of garlic [23], dose-dependently inhibits the release of $PGE_2$ from LPS-

activated RAW 264.7 cells ($IC_{50}$: 2.4 $\mu$M). The inhibitory effect of ajoene was due to a dose-dependent inhibition of COX-2 enzyme activity ($IC_{50}$: 3.4 $\mu$M). Ajoene did not reduce COX-2 mRNA or protein expression, but rather enhanced LPS-induced COX-2 without affecting the basal level of expression of COX-2.

Thus, ajoene was shown to inhibit prostaglandin synthesis by a mechanism common to non-steroidal anti-inflammatory drugs. As demonstrated for ajoene, NSAIDs were reported to increase COX-2 expression in rodent models [37–39]. Our own studies confirmed these observations: indomethacin (1 $\mu$M) as well as the COX-2-selective inhibitor NS-398 (20 $\mu$M) were found to increase COX-2 protein levels in LPS-activated RAW 264.7 cells. Since prostanoids are suggested to be negative feedback regulators of COX-2 expression in murine macrophages [37,40], inhibition of prostaglandin synthesis may lead to increased COX-2 expression as shown for ajoene.

Along with COX-2, another immediate-early gene, the inducible isoform of nitric oxide synthase, is expressed in LPS-activated murine macrophages. Numerous reports suggest a complex "cross-talk" between the COX-2 and iNOS pathways. Although not all published data are consistent, most findings indicate that nitric oxide seems to down-regulate COX-2 expression. Effective inhibition of NO synthesis by inhibitors of NOS activity such as $N^G$-monomethyl-L-arginine (L-NMMA) have been shown to increase COX-2 protein expression in rodent macrophages [41–44]. In addition, Habib *et al*. found increased COX-2 mRNA levels after treatment of LPS-activated rat peritoneal macrophages with L-NMMA and aminoguanidine [42].

Since we recently reported that ajoene reduces NO synthesis by inhibiting the induction of iNOS in LPS-activated RAW 264.7 cells [29], reduced NO levels may contribute to the observed enhanced COX-2 protein and mRNA expression after ajoene treatment. The ajoene concentration needed for an approximate 80% inhibition of nitrite accumulation in cell culture supernatants was 10 $\mu$M, the same dose for which we found increased COX-2 protein and mRNA expression. Similarly, indomethacin was shown to increase COX-2 protein expression at a concentration where it also inhibited iNOS protein expression and NO release from LPS-activated J774 murine macrophages [37]. Besides this possible interference of ajoene with negative feedback mechanisms of NO and prostanoids on COX-2 expression, other possibilities, particularly an effect of ajoene on COX-2 mRNA stability or effects on the transcriptional level, cannot be ruled out.

Earlier studies addressing the effect of ajoene on the metabolism of arachidonic acid showed that ajoene is also an inhibitor of COX-1 enzyme activity: Srivastava *et al*. [27] demonstrated that ajoene reduced thromboxane $A_2$ formation in washed platelets. However, the effective concentrations found were quite high ($IC_{50}$: 128 $\mu$M). In a cell-free system using a preparation from sheep seminal vesicle microsomes as a source of COX-1, ajoene was found to inhibit COX-1 activity with an $IC_{50}$ of 5.1 $\mu$M [28]. As $IC_{50}$ values vary greatly depending on the *in vitro* system used, comparisons of $IC_{50}$ values obtained from completely different systems (e.g. intact cells versus enzyme-based assay) are questionable. From the present data, it is therefore difficult to judge which isoform, COX-1 or COX-2, is preferentially inhibited by ajoene. Further studies using enzyme-based assays will clarify this question.

In summary, motivated by epidemiological data suggesting a chemopreventive effect of *allium* constituents especially for gastrointestinal carcinomas and interesting *in vitro* data available for the garlic-derived compound ajoene, we addressed the question as to whether this natural product might interfere with the COX-2 pathway. We found an activity and mechanism of action for ajoene resembling that of NSAIDs, which are thought to display a chemopreventive effect by their common ability to inhibit prostaglandin synthesis [6]. The presented data for ajoene might therefore at least in part contribute to a better understanding of the chemopreventive effect attributed to garlic or garlic preparations.

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