Journal of Enzyme Inhibition and Medicinal Chemistry, October 2004 Vol. 19 (5), pp. 431–436

Taylor & Francis
healthsciences

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Inhibition of 5-lipoxygenase and Leukotriene C_4 Synthase in Human Blood Cells by Thymoquinone

MAHMOUD MANSOUR[a,b,*] and SUSANNE TORNHAMRE[a]

[a]*Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II, Karolinska Institutet S 171 77, Stockholm, Sweden;*
[b]*Biochemistry Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt*

(Received 8 March 2004; In final form 18 June 2004)

Black cumin seed, *Nigella sativa L.*, and its oils have traditionally been used for the treatment of asthma and other inflammatory diseases. Thymoquinone (TQ) has been proposed to be one of the major active components of the drug. Since leukotrienes (LTs) are important mediators in asthma and inflammatory processes, the effects of TQ on leukotriene formation were studied in human blood cells. TQ provoked a significant concentration-dependent inhibition of both LTC_4 and LTB_4 formation from endogenous substrate in human granulocyte suspensions with IC_{50} values of 1.8 and 2.3 μM, respectively, at 15 min. Major inhibitory effect was on the 5-lipoxygenase activity (IC_{50} 3 μM) as evidenced by suppressed conversion of exogenous arachidonic acid into 5-hydroxy eicosatetraenoic acid (5HETE) in sonicated polymorphonuclear cell suspensions. In addition, TQ induced a significant inhibition of LTC_4 synthase activity, with an IC_{50} of 10 μM, as judged by suppressed transformation of exogenous LTA_4 into LTC_4. In contrast, the drug was without any inhibitory effect on LTA_4 hydrolase activity. When exogenous LTA_4 was added to intact or sonicated platelet suspensions preincubated with TQ, a similar inhibition of LTC_4 synthase activity was observed as in human granulocyte suspensions. The unselective protein kinase inhibitor, staurosporine failed to prevent inhibition of LTC_4 synthase activity induced by TQ. The findings demonstrate that TQ potently inhibits the formation of leukotrienes in human blood cells. The inhibitory effect was dose- and time-dependent and was exerted on both 5-lipoxygenase and LTC_4 synthase activity.

Keywords: *Nigella sativa*; Thymoquinone; 5-lipoxygenase; LTC_4 synthase; LTA_4 hydrolase; Anti-inflammatory

INTRODUCTION

The seeds of *Nigella sativa*, a member of the Ranunculaceae family, are commonly known as black seed. In folk medicine, the black seeds and their derivatives, mainly oils, have traditionally been used for the treatment of bronchial asthma[1] as well as for local external treatment for relief of pain and stiffness in the joints.[2] This indicates that the oil might have effects on inflammatory mediators associated with these diseases. In agreement with this, several studies report such activities for *Nigella sativa L.* and its extracts.[3–6] Chemically the seeds contain fixed oil (30–33% w/w) and a volatile oil (0.43–0.73% w/w).[7] The volatile oil has been shown to contain 18–24% thymoquinone (TQ) and monoterpens, mainly p-cymene and α-pinene (32% and 9.3% respectively).[8] Also the fixed oil fraction contains a not negligible amount of TQ, although in low concentration (0.15%).[2] We and other have previously demonstrated that this major component TQ is a potent superoxide radical scavenger in different assays.[9,10] In addition non-enzymatic inhibition of lipid peroxidation has been reported in ox-brain phospholipid liposomes- and rat heart homogenate -incubations in the presence of TQ.[2,11] *In vivo* pre-treatment with oral TQ in the rat protected against oxidative damage induced by different free radical generating agents, including doxorubicin-induced cardiotoxicity[11] and carbon tetrachloride-provoked hepatotoxicity.[12] Moreover, Badary *et al.*[13] showed that TQ attenuated nephrotoxicity induced by cisplatin and potentiated its antitumor activity. The antitumor activity of TQ has been indicated by inhibition of benzo(a)pyrene-induced stomach carcinogensis in mice.[14] In addition, the fixed oil of *Nigella sativa* and TQ have also been reported to induce inhibition of thromboxane and 5-lipoxygenase products in rat peritoneal leukocytes stimulated with calcium ionophore (A23187).[2,15]

*Corresponding author. Tel: +20-2-508-42-37. Fax: +20-2-2633996. E-mail: mansour1960us@yahoo.com

ISSN 1475-6366 print/ISSN 1475-6374 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/14756360400002072

RIGHTSLINK

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Leukotrienes (LTs) are a family of oxygenated metabolites of arachidonic acid, synthesized by 5-lipoxygenase enzyme.[16] They are formed predominantly by inflammatory cells like polymorphonuclear leukocytes, macrophages and mast cells. During cell activation, leukotriene biosynthesis is initiated by translocation of cytosolic phospholipase A_2 and 5-lipoxygenase to the nuclear envelope and liberation of arachidonic acid.[17,18] The liberated arachidonic acid binds to arachidonate transfer protein, 5-lipoxygenase-activating protein (FLAP) which facilitates presentation to 5-LO for conversion to the epoxide LTA_4. The further metabolism of LTA_4 to the biologically active leukotrienes proceeds via two different routes. LTA_4 may either be utilized by LTA_4 hydrolase to produce the potent neutrophil chemo-attractant LTB_4[19] or by membrane bound LTC_4 synthase that catalyses the conjugation of LTA_4 with the tripeptide glutathione, to form the asthma mediator LTC_4.[20] Once formed, LTC_4 is exported out of the cell facilitated by transporters such as the multidrug resistance associated protein (MRP1).[21] The subsequent conversion of LTC_4 to LTD_4 via removal of the γ-glutamyl moiety from glutathione is catalysed by γ -glutamyl transpeptidase, an enzyme located at the external site of the plasma membrane. The next metabolic step results in substantial loss of the biological activity, through the removal of glycine resulting in LTE_4 formation.[22] The cysteinyl-LTs bind to specific receptors and mediate a wide variety of inflammatory responses.[23] In the airway system, LTC_4 is one of the relevant mediators involved in bronchial asthma and is responsible for many of the observed cardinal symptoms of the disease such as bronchoconstriction, increased mucus secretion and edema formation. Cysteinyl-LTs antagonists are used clinically in asthma control.[24]

A key proinflammatory role has been postulated for LTB_4 in its ability to recruit and activate inflammatory cells.[25] It stimulates the production of many pro-inflammatory mediators including cytokines,[19] the release of lysosomal enzymes,[26] the generation of superoxide radicals in neutrophils[27] and hydrogen peroxide in human monocytes as well as activates NADPH oxidase of human eosinophils.[28,29] These effects indicate an ability of LTB_4 to accentuate free radical generation and prolong tissue inflammation. In the light of the reported anti-inflammatory activities of TQ and effect on eicosanoid generation in leukocytes, it was of interest to investigate the effects of TQ on different enzyme-activities involved in leukotriene-synthesis. Thus, in the present study we investigated the effect of TQ on leukotrienes and 5-HETE formation from endogenous and exogenous substrate in intact and sonicated human granulocyte suspensions and the effect of TQ on LTC_4 synthase activity in human platelets.

MATERIALS AND METHODS

Materials

Vacutainer® blood collection tubes were purchased from Becton Dickinson (Rutherford, NJ. USA) and sodium metrizoate (Lymphoprep®) was from Nyegaard and Co (Oslo, Norway). Ionophore A23187 was obtained from Calbiochem-Boehring (La Jolla, CA. USA). Leukotriene A_4 methyl ester was a generous gift from Dr. Robert Zipkin, Biomol Research laboratories (Plymouth Meeting, PA. USA) and was saponified as described[30]. Leukotriene B_4 and C_4 were purchased from Biomol Research Laboratories (Plymouth Meeting, PA. USA). Thymoquinone (TQ) and fatty acid-free human serum albumin (HSA) were purchased from Sigma Chemical Co. (St. Louis, MO. USA).

Preparation of Cell Suspension and Sonicates

Peripheral venous blood from healthy volunteers was collected in EDTA-containing Vacutainer® blood collection tubes. After centrifugation at 200*g* for 15 min, the platelet rich plasma was removed and the granulocyte fraction was isolated according to standard laboratory technique.[31] Briefly, equal amount of 2% dextran T500 (in saline) was added and then allowed to stand for 30 min at 4°C to sediment erythrocytes. The leukocyte-rich upper layer was centrifuged at 280*g* for 10 min. The resultant leukocyte pellet was washed twice with PBS, and resuspended in hypotonic ammonium chloride (0.16 M NH_4Cl, 17 mM tris/HCl pH 7.4) for 30 min to lyse any remaining erythrocytes. After centrifugation at 280*g* for 10 min, the cells were suspended in PBS, and Lymphoprep® was carefully added under the cell suspension and centrifuged for 40 min at 400*g*. The obtained polymorphonuclear granulocyte pellet was washed and suspended in PBS. The cells were counted in a Bürker chamber and a final granulocyte count was adjusted to 15×10^6 cells/ml. The granulocyte viability was about 96% as judged by the trypan blue exclusion test.

The platelet suspensions were prepared as follows: The blood was centrifuged at 200*g* for 15 min and the platelet-rich plasma was collected. Thereafter, the platelet rich plasma was further centrifuged at 650*g* for 20 min, the platelets were washed twice in 0.15 M NaCl buffered with 12 mM tris/HCl, pH 7.4 containing 1.5 mM EDTA. Thereafter the platelets were resuspended in phosphate buffer saline (PBS; 0.9 mM Ca^{2+}, pH 7.4) to a final concentration of 400×10^6 platelets/ml.

For experiments with granulocyte or platelet sonicates, cells were sonicated (Ultrasonic disintegrator Mk2; power output, 50–150 W) in PBS without calcium in the presence of 1 mM EDTA at

RIGHTSLINK

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

0°C for 3 × 5 s. Prior to incubation 2 mM $CaCl_2$, 0.3 mg human serum albumin/ml and 1 mM ATP were added to the granulocyte sonicates and 2 mM $CaCl_2$ and 4 mM glutathione were added to the platelet sonicates.

Incubation Procedure

Intact or sonicated human granulocyte suspensions (1 ml, 15 × 10^6 cells/ml) were preincubated at 37°C for 15 min with or without 1–100 μM of TQ. Thereafter 10 μM LTA_4, 1 μM A23187 or 20 μM AA was added. Reactions were terminated after 5 min by adding 5 vol of ethanol (99%).

Intact or sonicated human platelet suspensions (1 ml, 400 × 10^6 cells/ml) were equilibrated at 37°C for 2 min with or without staurosporine prior to preincubation with or without 1–100 μM TQ. Thereafter the platelets were incubated for 5 min in the presence of exogenous LTA_4. The production of LTC_4 was terminated by addition of 5 vol ethanol.

Purification, Identification and Quantitation of Leukotrienes and Monohydroxy Acids

The samples dissolved in ethanol, were centrifuged and the clear supernatants were evaporated to dryness. The obtained residues were dissolved in the HPLC mobile phase, and then centrifuged before injection. Identification and quantitation of LTs were performed by RP-HPLC using a Nova-Pak C_{18} column (3.9 × 150 mm, Water Associates, Milford, MA. USA) eluted with acetonitrile/methanol/water/acetic acid (27:18:54:0.8 by vol., apparent pH 5.6) for leukotriene analysis or methanol/water/acetic acid (73:27:0.01 by vol.) for mono-HETEs analysis at flow rate of 1 ml/min and a variable wavelenght UV-detector (LDC Spectromonitor III) at 280 nm (LTs) or 236 nm (Mono-HETEs), connected to an integrator (EZ ChromTM Chromatography data system).

Statistical Analysis

Results are expressed as mean ± SEM. Statistical comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Significance was accepted at $P < 0.05$.

RESULTS

Effects of TQ on A23187-induced Leukotrienes Formation in Human Granulocyte Suspensions

Human granulocyte cell suspensions were stimulated with 1 μM A23187 for 5 min. In control incubations (without TQ) 95.5 ± 8.2 (mean ± SEM, $n = 5$) pmol LTC_4/ml and 488 ± 88.5 ($n = 5$) pmol LTB_4/ml were formed. Preincubation of human granulocyte cells with different concentrations of TQ (1,3,10 and 100 μM) for 15 min, prior to stimulation with A23187 for another 5 min, led to significant reduction in LTC_4 and LTB_4 formation (Figure 1). The inhibitory effect of TQ was found to be concentration-dependent as compared with diluent treated control cells. The IC_{50} of TQ for LTC_4 and LTB_4 formation were 1.8 and 2.3 μM respectively.

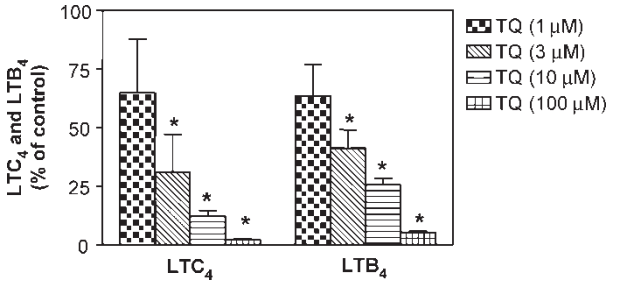

FIGURE 1 Effects of TQ on leukotrienes C_4 and B_4 formation in human granulocyte suspensions stimulated with A23187. Human granulocyte cells (15 × 10^6 cells/ml) were pre-incubated at 37°C with different concentrations of TQ (1–100 μM) for 15 min. Thereafter, the cells were stimulated with A23187 (1 μM) for 5 min. The formation of LTC_4 and LTB_4 are expressed relative to the formation in control incubations without TQ. LTs were identified and quantified by RP-HPLC. Each value represents the mean of 3–4 experiments performed in duplicate. Error bars indicate standard error of the mean. *Significantly different from the control, $P < 0.05$.

Effects of TQ on LTA_4 Hydrolase and LTC_4 Synthase Activity in Intact Human Granulocytes

The effect of TQ (1,3,10 and 100 μM) on LTA_4 hydrolase and LTC_4 synthase activity in intact granulocytes was also investigated. In control incubations (without TQ) exogenously added LTA_4 (10μM) was converted to 154.5 ± 72 (n = 4) pmol LTC_4/ml and 476 ± 29 (n = 4) pmol LTB_4/ml. Pre-treatment of granulocyte cells with different concentrations of TQ for 15 min prior to incubation with exogenous LTA_4 inhibited the LTC_4 synthase activity in a concentration-dependent manner (Figure 2) with an IC_{50} of approximately 10μM. In contrast, the LTA_4 hydrolase was not inhibited by TQ (1-100μM). The LTB_4 formation tended to be enhanced reaching significant levels at 10 and 100 μM TQ (Figure 2).

Effect of TQ on 5-lipoxygenase Activity after Incubation of Sonicated Human Granulocyte with Exogenous Arachidonic Acid

Human granulocyte sonicates were incubated in the presence of ATP (1mM) with or without different concentrations of TQ for 15 min prior to incubation

RIGHTSLINK

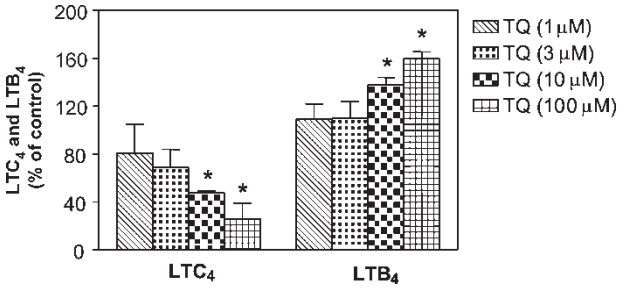

FIGURE 2 Effects of TQ on leukotriene C_4 synthase and leukotriene A_4 hydrolase activity in human granulocyte suspensions incubated with exogenous LTA_4. Human granulocyte cells (15×10^6 cells/ml) were pre-incubated at 37°C for 15 min with different concentrations of TQ (1–100 μM), prior to incubation with LTA_4 (10 μM) for 5 min. The formation of LTC_4 and LTB_4 are expressed relative to formation in the control incubations without TQ. Each value represents the mean of 3–4 experiments performed in duplicate. Error bars indicate standard error of the mean. *Significantly different from the control, $P < 0.05$.

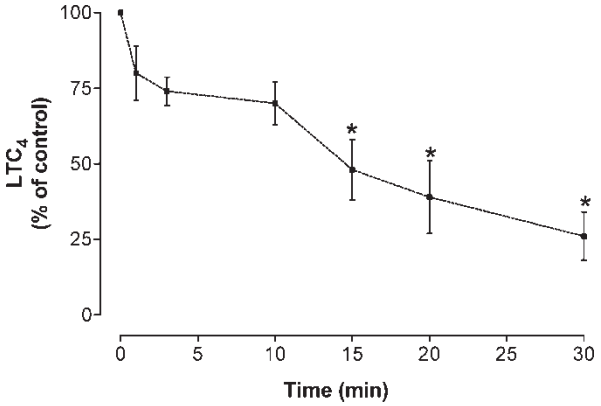

FIGURE 4 Time-course of the effect of TQ on leukotriene C_4 synthase activity in human platelets. Human platelet suspensions were preincubated at 37°C for 1–30 min with or without 10 μM TQ prior to incubation with exogenous LTA_4 (10 μM) for 5 min. Values are expressed relative to LTC_4 formation in the control incubations without TQ. Each value represents the mean of 3–4 experiments performed in duplicate. Error bars indicate standard error of the mean. *Significantly different from the control, $P < 0.05$.

for another 10 min with 20μM arachidonic acid. In control incubations (without TQ) 436 ± 85 (n = 4) pmol 5-HETE/ml was formed. In the presence of different concentrations of TQ (3, 10, 30 and 100 μM) a concentration-dependent attenuation of 5-HETE formation was observed with approximately 50% inhibition at 3μM (Figure 3).

Effect of TQ on LTC_4 Synthase Activity in Human Intact or Sonicated Platelets

After addition of exogenous LTA_4 (10μM) to the human platelet suspension for 5 min, the formation of 710 ± 82 pmol LTC_4 / ml was identified.

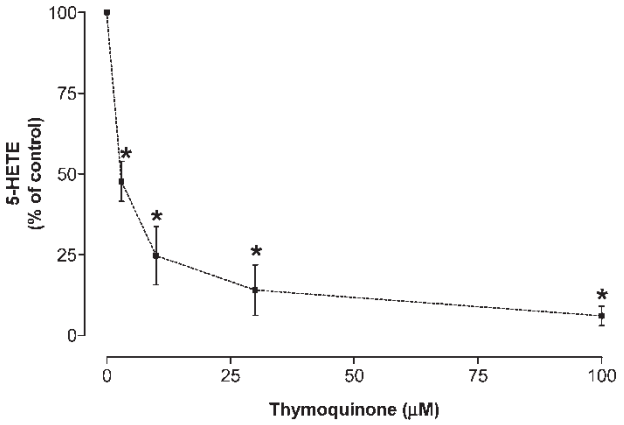

FIGURE 3 Effect of TQ on 5-lipoxygenase activity in human granulocyte sonicates incubated with exogenous AA. Human granulocyte sonicates were pre-incubated at 37° for 10 min with different concentrations of TQ (3,10,30 and 100 μM) prior to incubation for 10 min with arachidonic acid (20 μM) in the presence of ATP (1 mM). The formation of 5-HETE is expressed relative to formation in control incubations without TQ. Each value represents the mean of 4 experiments performed in duplicate. Error bars indicate standard error of the mean. *Significantly different from the control, $P < 0.05$.

Time course studies revealed that TQ inhibited the LTC_4 synthase activity in a time-dependent manner. Human platelet suspension was pre-incubated with 10 μM TQ for various times (1–30 min) prior to incubation with exogenous LTA_4 (10 μM) for 5 min, Inhibition of LTC_4 formation by TQ increased from 20 ± 8 to 73 ± 9% when the pre-incubation time was increased from 1 to 30 min (Figure 4).

Preincubation with different concentrations of TQ (1,3,10 and 100 μM) for 15 min prior to addition of exogenous LTA_4 inhibited platelet LTC_4 synthase activity in a concentration-dependent manner and significant inhibition of LTC_4 formation was found at 10 and 100 μM of TQ (Figure 5A). The IC_{50} value was approximately 9 μM.

Similar inhibition of the LTC_4 synthase activity was obtained in sonicated platelet suspensions

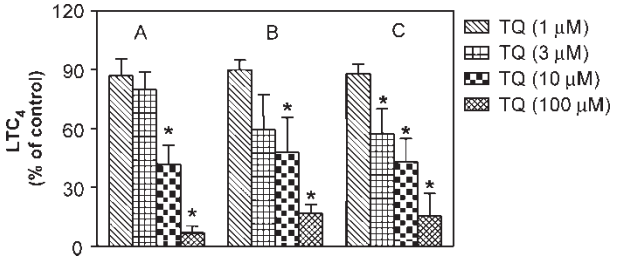

FIGURE 5 Effect of TQ on leukotriene C_4 synthase activity in intact, sonicated or staurosporine-treated platelet suspensions. Human platelet suspension (A), sonicated platelets (B) and platelet suspensions treated for 2 min with 1 μM staurosporine (C) were preincubated at 37°C for 15 min with and without different concentration of TQ (1–100 μM) prior to incubation with exogenous LTA_4 (10 μM). Values are expressed relative to LTC_4 formation in control incubations without TQ. Each value represents the mean of 3–4 experiments performed in duplicate. Error bars indicate standard error of the mean. *Significantly different from the control, $P < 0.05$.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

RIGHTSLINK

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

preincubated with the selected concentrations of TQ (Figure 5B).

In a subsequent experiment, intact platelet suspensions were incubated with the non- selective protein kinase C inhibitor, staurosporine (1 μM) for 2 min before addition of different concentrations of TQ. No prevention of the TQ-induced inhibition of LTC_4 synthase activity in human platelet suspensions could be detected in the presence of 1 μM staurosporin. A significant inhibition of LTC_4 formation was observed with 3, 10 and 100 μM TQ (Figure 5C).

DISCUSSION

In the present investigation, thymoquinone exhibited a potent inhibitory effect on the formation of 5-lipoxygenase products after A23187 stimulation. TQ inhibited both the formation of LTC_4 and LTB_4 with IC_{50} values of 1.8 and 2.3 μM respectively (Figure 1). These results are in line with previous studies reporting that TQ inhibited 5-lipoxygenase enzyme in rat peritoneal leukocytes[2] and rat polymorph-nuclear leukocyte (PMNL)[15] stimulated with A23187.

To elucidate the inhibitory effects of TQ on leukotriene generation, we studied its effect on different enzyme activities involved in leukotriene synthesis. LTA_4 is the substrate for both LTC_4 synthase and LTA_4 hydrolase of human granulocytes. Pre-incubation of human granulocytes with TQ for 15 min prior to incubation with exogenous LTA_4, inhibited LTC_4 generation (IC_{50} 10 μM), in parallel with a significant increase in LTB_4 formation (Figure 2). The inability of the drug to inhibit the conversion of LTA_4 to LTB_4 excludes an inhibitory effect on LTA_4 hydrolase. The increased LTB_4 formation could be explained with increased availability of the substrate due to the inhibition of LTC_4 synthase activity. These results revealed inhibition of LTC_4 synthase and an additional more potent inhibitory effect on LTA_4 formation.

To further investigate whether TQ exerts this effect on 5-lipoxygenase, 5-lipoxygenase activating protein (FLAP) or phospholipase A_2 level, sonicated human granulocytes were incubated with exogenous arachidonic acid.

Pre-incubation with TQ induced a significant dose-dependent inhibition (IC_{50} 3 μM) on 5-HETE formation. This indicates that TQ potently inhibits 5-lipoxygenase activity in human granulocytes, since FLAP is not needed for 5-lipoxygenase activity in sonicated human granulocytes. However, an additional inhibitory effect at phospholipaseA_2 level cannot be excluded. Inhibition of 5-lipoxygenase was not specific since TQ also inhibits 12- and 15-lipoxygenase as evidenced by decreased transformation of arachidonic acid into 12-HETE and 15-HETE (data not shown). These results confirm previous results demonstrating inhibition of 5-HETE formation from rat PMNL stimulated with A23187 by TQ.[15]

The inhibitory effect of TQ on lipoxygenase enzymes are not due to non-specific cytotoxicity, as confirmed by the failure of the granulocyte cells to take up trypan blue after incubation with TQ. The lipoxygenase reactions involve a free radical mechanism and TQ possesses the structural element typical for an antioxidant effect. In previous *in vitro* studies we have reported that TQ is a potent superoxide free radical scavenger, being as effective as superoxide dismutase against superoxide radical and has antioxidant activity in lipid peroxidation.[9] Moreover, TQ inhibits *in vitro* lipid peroxidation induced by Fe^{3+}/ascorbate using rat liver homogenates, the IC_{50} value for TQ scavenging property being in the micromolar range.[12,32] In addition Burits and Bucar[33] demonstrated that TQ showed a respectable radical scavenging property against free radicals. It could be concluded that TQ inhibits lipoxygenase enzymes due to their antioxidant function. It may therefore be classified as a redox 5-lipoxygenase inhibitor.

However the present study indicates that TQ also has additional effects on LTC_4 synthase activity, as it provoked an inhibitory effect on the enzyme activity in human granulocytes and platelets, judged by a significant decrease in LTC_4 formation from LTA_4. Since platelets lack LTA_4 hydrolase an eventual effect on this enzyme affecting the availability of the common substrate, is excluded.

It has been shown that LTC_4 synthase contains two consensus sequences for protein kinase C phosphorylation.[34] Phosphoregulation of LTC_4 synthase activity via a protein kinase C (PKC)-dependent mechanism has been reported in normal human granulocytes,[30] human platelets[30] as well as in human leukemic cell lines.[35] Direct activation of PKC with nanomolar concentration of 4-phorbol-12-myristate-13-acetate (PMA) inhibited the production of LTC_4 in human granulocytes[30] and platelets.[30] We have earlier reported that, the rather non-specific protein kinase inhibitor, staursporine prevented PMA-induced suppression of LTC_4 formation in human platelets pre-incubated with LTA_4.[30] This indicating that transformation of LTA_4 into LTC_4 in human platelets could be controlled via phosphoregulation of LTC_4 synthase. To elucidate whether the inhibition of LTC_4 synthase induced by TQ is mediated via this phosphoregulatory mechanism, staurosporine was added. However, staursporine failed to prevent the inhibitory effect of TQ on LTC_4 synthase activity. In addition TQ exerted a similar inhibitory effect on LTC_4 synthase activity in sonicated platelets as demonstrated on LTC_4

RIGHTSLINK

synthase in intact platelets. These results indicate that TQ displays a direct inhibitory effect on LTC_4 synthase rather than interfering with signal transduction in human blood cells.

Both inhibitions on the 5-lipoxygenase (probably due to its scavenger effect) and on the LTC_4 synthase activity, are in agreement with the traditional use of TQ in asthma and as an anti-inflammatory agent. It might also explain results from earlier studies reporting the relaxant effects of extracts from *Nigella sativa L.* on methacholine-induced contraction of the tracheal smooth muscles of guinea pigs[6] and protection against histamine-induced bronchospasm without affecting histamine H_1 receptors.[36]

The traditional use of *Nigella sativa* seed in the treatment of bronchial asthma might be due to inhibition of LTC_4 synthase activity and additional effects on 5-lipoxygenase exerted by TQ. The IC_{50} on LTC_4 formation in granulocyte suspension was 1.8 μM from endogenous substrate and 9 μM in platelet suspensions incubated with LTA_4 for 15 min and decreased at longer pre-incubation times. However to our knowledge the actual concentration of TQ or its metabolites in human blood after ingestion of *Nigella sativa* seeds has not been measured.

In conclusion, the present results demonstrate that TQ dose-dependently inhibits leukotriene formation in human blood cells, both on the 5-lipoxygenase and LTC_4 synthase activity. The findings contribute to elucidate the mechanism of action of *Nigella sativa* seeds and TQ as anti-inflammatory agents, since leukotrienes are known to play a pivotal role in the inflammatory process.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Acknowledgements

We thank Dr Robert Zipkin, Biomol Research Laboratories (Plymouth Meeting, PA, USA) for the generous gift of LTA_4 methyl ester. This project was supported by the Research Funds of Karolinska Institutet.

References

[1] Sayed, M. (1980) *J. Ethnopharmacol.* **2**(1), 19–22.
[2] Houghton, P., Zarka, R., de las Heras, B. and Hoult, J. (1995) *Planta Med.* **61**(1), 33–36.
[3] Al-Ghamdi, M. (2001) *J. Ethnopharmacol.* **76**(1), 45–48.
[4] Chakravarty, N. (1993) *Ann. Allergy* **70**(3), 237–242.
[5] Haq, A., Abdullatif, M., Lobo, P., Khabar, K., Sheth, K. and Al-Sedairy, S. (1995) *Immunopharmacology* **30**(2), 147–155.
[6] Boskabady, M.H. and Shahabi, M. (1997) *Irn. J. Med. Sci.* **22**, 133–136.
[7] Gad, A. and El-Dakhakhny, M. (1963) *Planta Med.* **11**(2), 134–138.
[8] Al-Jassir, M. (1992) *Food Chem.* **45**, 239–243.
[9] Mansour, M.A., Nagi, M.N., El-Khatib, A.S. and Al-Bekairi, A.M. (2002) *Cell Biochem. Funct.* **20**(2), 143–151.
[10] Kruk, I., Michalska, T., Lichszteld, K., Kladna, A. and HY, A.E. (2000) *Chemosphere* **41**(7), 1059–1064.
[11] Nagi, M. and Mansour, M. (2000) *Pharmacol. Res.* **42**(3), 283–289.
[12] Mansour, M. (2000) *Life Sci.* **66**(26), 2583–2591.
[13] Badary, O., Nagi, M., Al-Shabanah, O., Al-Sawaf, H., Al-Sohaibani, M. and Al-Bekairi, A. (1997) *Can. J. Physiol. Pharmacol.* **75**, 1356–1361.
[14] Badary, O., Al-Shabanah, O., Nagi, M., Al-Rikabi, A. and Elmazar, M. (1999) *Eur. J. Cancer Prev.* **8**(5), 435–440.
[15] El-Dakhakhny, M., Madi, N.J., Lembert, N. and Ammon, T. (2002) *J. Ethnopharmacol.* **81**, 161–164.
[16] Samuelsson, B. (1987) *Drugs* **33**, 2–9.
[17] Pouliot, M., Mcdonald, P.P., Krump, E., Mancini, J.A., McColl, S.R., Weech, P.K. and Borgeat, P. (1996) *Eur. J. Biochem.* **238**, 250–258.
[18] Petersgolden, M. (1998) *Am. J. Respir. Crit. Care Med.* **227**, S227–S232.
[19] Ford-Hutchinson, A.W. (1990) *Crit. Rev. Immunol.* **10**, 1–12.
[20] Lam, B.K. and Austen, F.K. (2000) *Am. J. Respir. Crit. Care Med.* **161**, S16–S19.
[21] Jedlitschky, G. and Keppler, D. (2002) *Vitam. Horm.* **64**, 153–184.
[22] Keppler, D. (1992) *Rev. Physiol. Biochem. Pharmacol.* **121**, 1–30.
[23] Hui, Y. and Funk, C. (2002) *Biochem. Pharmacol.* **64**, 1549–1557.
[24] Claesson, H. and Dahlén, S. (1999) *J. Int. Med.* **245**, 205–227.
[25] Henderson, W.J. (1994) *Ann. Intern. Med.* **121**, 684–697.
[26] Palmblad, J., Malmsten, C.L., Udén, A.M., Rådmark, O., Engstedt, L. and Samuelsson, B. (1981) *Blood* **58**, 658–661.
[27] Serhan, C.N., Radin, A., Smolen, J.E., Korchak, H., Samuelsson, B. and Weissmann, G. (1982) *Biochem. Biophys. Res. Commun.* **107**, 1006–1012.
[28] Crooks, S.W. and Stockley, R.A. (1998) *Int. J. Biochem. Cell Biol.* **30**, 173–178.
[29] Bankers-Fulbright, J., Kita, H., Gleich, G. and O'Grady, S. (2001) *J. Cell Physiol.* **189**, 306–315.
[30] Tornhamre, S., Edenius, C. and Lindgren, J.Å. (1995) *Eur. J. Biochem.* **234**, 513–520.
[31] Böyum, A. (1968) *J. Clin. Lab. Investig.* **97**, 77–89.
[32] Badary, O., Taha, R., Gamal-elDin, A. and Abdel-Wahab, M. (2003) *Drug Chem. Toxicol.* **26**, 87–96.
[33] Burits, M. and Bucar, F. (2000) *Phytother. Res.* **14**, 323–328.
[34] Lam, B.K., Penrose, J.F., Freeman, G.J. and Austen, K.F. (1994) *Proc. Natl Acad. Sci. USA* **91**, 7663–7667.
[35] Ali, A., Ford-Hutchinson, A.W. and Nicholson, D.W. (1994) *J. Immunol.* **153**, 776–788.
[36] Mahfouz, M. and El-Dakhakhny, M. (1960) *Egypt Pharm. Bull.* **42**, 411–424.

RIGHTSLINK