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European Journal of Pharmacology 506 (2004) 189–197

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Formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in carrageenan-induced air pouch model in rats

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Received 13 May 2004; received in revised form 13 October 2004; accepted 28 October 2004

Abstract

To investigate a possible role of 8-isoprostaglandin $F_{2\alpha}$ in inflammation, 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels were determined by enzyme immunoassay (EIA) in carrageenan-induced air pouch model in rats. In this model, 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels were found to be increased significantly. To evaluate whether this increase was due to the development of inflammation or solely to cyclooxygenase-2 induction, a lipopolysaccharide-induced air pouch model, in which only cyclooxygenase-2 induction occurs without inflammation, was used. In this model, 8-isoprostaglandin $F_{2\alpha}$ was also found to be increased parallel to the increase in prostaglandin E_2 level. Cyclooxygenase-dependent formation of 8-isoprostaglandin $F_{2\alpha}$ was investigated in carrageenan-induced air pouch model by administrating nonselective cyclooxygenase inhibitor indomethacin, selective cyclooxygenase-1 inhibitor valeryl salicylate or selective cyclooxygenase-2 inhibitor SC-582368 (4-(5-(4-chlorophenyl)-3-3-trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonanmide) 1 h before carrageenan injection. All these inhibitors significantly inhibited the production of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2. These findings show that 8-isoprostaglandin $F_{2\alpha}$ can be formed in carrageenan-induced air pouch model in rats. The formation of 8-isoprostaglandin $F_{2\alpha}$ in lipopolysaccharide-induced air pouch model and the inhibition of its production by various cyclooxygenase inhibitors provide evidence for cyclooxygenase-dependent formation of isoprostanes in this model.

Keywords: 8-Isoprostaglandin $F_{2\alpha}$; Prostaglandin E_2; Carrageenan-induced air pouch model; Cyclooxygenase inhibitor; Vitamin E

1. Introduction

Inflammation is a complex process that manifests itself with vascular and cellular components. Many different types of mediators are believed to participate in the inflammation process. Among these mediators, prostaglandins are one of the eminent one. Prostaglandins have distinct effects on inflammation. Prostaglandin E_2 and prostacyclin produce vasodilatation, especially in arterioles, metaarterioles, precapillaries and venules (Greenberg and Sparks, 1969). They cause augmentation of vascular permeability in synergy with other mediators, such as histamine and bradykinin (Williams, 1979). Prostaglandins can also cause pain (Ferreira, 1972) and fever (Feldberg and Saxena, 1971). As prostaglandins reproduce the cardinal signs of inflammation, it is generally believed that they are mediators of acute inflammation.

Isoprostanes are a group of compounds produced from arachidonic-acid-like prostaglandins. The most prominent difference between prostaglandins and isoprostanes is the occurrence of isoprostanes via a cyclooxygenase-independent, free-radical-catalyzed mechanism. Isoprostanes have different effects on various systems. They cause renal vasoconstriction and reduce glomerular filtration rate and renal blood flow in rats (Takahashi et al., 1992). They also cause shape change and calcium release from intracellular stores in human platelets (Morrow et al., 1992; Pratico et al., 1996). The quantitation of isoprostanes in urine, plasma, tissue and other biological fluids has been

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doi:10.1016/j.ejphar.2004.10.050

extensively used as a reliable marker of in vivo lipid peroxidation. Although free-radical-induced isomers of other prostaglandins, such as thromboxanes and leukotrienes, have been reported (Morrow et al., 1996; Harrison and Murphy, 1995), the most extensively studied compounds are isomers of prostaglandin $F_{2\alpha}$ and are called F_2-isoprostanes. Increased levels of F_2-isoprostanes in body fluids, such as urine, blood, cerebrospinal fluid and pericardial fluid, as a clinical marker of lipid peroxidation were shown in many clinical settings, such as diabetes mellitus (Gopaul et al., 1995; Davi et al., 1999), hypercholesterolemia (Reilly et al., 1998), coronary angioplasty (Reilly et al., 1997) and Alzheimer's disease (Pratico and Delanty, 2000).

Although isoprostanes are believed to occur via cyclooxygenase-independent mechanisms, there are studies indicating that they can also be formed by a cyclooxygenase-dependent mechanism. In this context, it has been reported that 8-isoprostaglandin $F_{2\alpha}$ could be formed as a minor product of the cyclooxygenase-1 enzyme in platelets and of the cyclooxygenase-2 isoform in monocytes (Pratico et al., 1995; Pratico and Fitzgerald, 1996). However, the administration of an aspirin regimen, designed to inhibit platelet cyclooxygenase-1, failed to suppress the elevated urinary 8-isoprostaglandin $F_{2\alpha}$ level (Reilly et al., 1996).

The aim of the present study is to investigate the formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in an acute inflammation model (carrageenan-induced air pouch model) in rats and to assess the effect of various cyclooxygenase inhibitors and vitamin E on the formation of 8-isoprostaglandin $F_{2\alpha}$ in this model.

2. Materials and methods

2.1. Animals

The experiments were performed on female Wistar albino rats weighing 130–200 g. The experiment was approved by Ankara University Animal Ethical Committee and was conducted according to the European Community guidelines for the use of experimental animals. The animals were kept at room temperature. All the groups, except the vitamin E group, were fed with their ordinary diet and allowed to drink water ad libitum.

2.2. Carrageenan-induced air pouch formation

The air pouch was formed by initial subcutaneous injection of 20 ml air into the back and successive injections of 10 ml air every 3 days to sustain its patency (Sedgwick and Lees, 1986). Seven days after the initial injection, 2 ml of 1% carrageenan solution in sterile 0.9% saline was injected into the cavity. Twelve hours after the carrageenan injections, the animals were anesthetised with ether, and 1 ml of heparinised saline (10 units/ml) was given to wash out the cavity.

2.3. Lipopolysaccharide-induced air pouch formation

An air cavity was produced as described above (see Carrageenan-induced air pouch formation). Seven days after the initial injection of air, 2 ml of lipopolysaccharide (1 μg/ml; *Escherichia coli* strain O111:β4) in saline was injected into the cavity. After 3 h of lipopolysaccharide treatment, 2 ml of 100 μM arachidonic acid was injected into the pouch; 15 min later, the animals were anesthetised with ether, and 1 ml of heparinised saline (10 units/ml) was given to wash out the cavity (Smith et al., 1998).

2.4. Collection and processing of exudates

The pouch cavity was opened, and the exudate was harvested. The exudate was transferred to ice-cold tubes and BW 755C (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride; 10^{-5} M final concentration), both cyclooxygenase and lipoxygenase inhibitor were added immediately to prevent the in vitro production of these metabolites of arachidonic acid. Cell-free exudate was achieved by centrifugation at 3000×*g*, 4 °C for 15 min, and was stored at −80 °C for the quantitation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2.

2.5. Extraction of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2

After the samples were thawed at room temperature, 1 ml of exudate was taken into a clean tube. $[H]^3$ prostaglandin F_2 (40 000 dpm) was added for calculating recovery during the extraction procedure. For the precipitations of proteins, 3 ml ice-cold ethanol (3×volume of the sample aliquot) was added to the sample and vortexed. The samples were allowed to cool for 5 min at −20 °C, then centrifuged at 4500×*g*, 4 °C, for 10 min to remove the precipitated proteins. The supernatant was poured into a clean tube, then the pH of the sample was adjusted to ~4.0 using glacial acetic acid. Ethanol was evaporated by vacuum centrifugation (MaxiDry Plus, Heto Holten, Allerod, Denmark). Extraction was performed using solid phase C18 and silica cartridge successively. One-milliliter aliquots were extracted on SepPak™ C18 (100 mg; Waters associates, Milford, MA), which had been preconditioned with 1 ml methanol and 2 ml ultrapure water. Then, the sample was passed in the cartridge. The cartridge was washed with 1 ml ultrapure water, followed by 1 ml hexane, and was eluted with 5 ml ethyl acetate containing 1% methanol. Hexane (1 ml) was added to the eluent, which was collected from C18 cartridges, and passed SepPak™ Silica (100 mg; Waters associates), which were preconditioned with 1 ml ethylacetate and 1 ml hexane–ethylacetate (1/1) successively. Eluent was collected with 1 ml methanol–ethylacetate (20:80). After evaporation, the

dried extract was reconstituted in a 1-ml enzyme immunoassay (EIA) buffer and stored at −80 °C until analysis.

2.6. Enzyme immunoassay of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2

8-Isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 was measured by EIA (Pradelles et al., 1985). The assay was performed in a total volume of 150 μl, with the following components being added in 50-μl volumes: standards or biological samples, enzymatic tracer and specific antiserum. After overnight incubation at 4 °C, the plates were washed, and 200 μl Ellman's reagent was dispensed into each well. After 1–2 h, the absorbance at 414 nm of each well was measured. Standard curve from 125 to 0.98 pg/ml was used to evaluate the concentrations of 8-isoprostaglandin $F_{2\alpha}$, and a standard curve from 62.5 to 0.48 pg/ml was used to determine prostaglandin E_2. Results were calculated by using the nonlinear regression of a four-parameter logistic model. Results were corrected according to the individual recovery of samples.

2.7. Measurement of plasma α-tocopherol

Plasma α-tocopherol was assayed by using high-pressure liquid chromatography (HPLC) with ultraviolet detection (Ohrvall et al., 1993). Briefly, 500 μl of plasma was extracted with 500 μl of ethanol containing 0.05 g/l of butylated hydroxytoluene and 2 ml of hexane. Supernatant (20 μl) was injected into an HPLC column (SGE SS Wakosil II 5C18RS, 5 μm; 250×4.6 mm). Diode Array detector was measured with wavelength of 220 nm.

2.8. Measurement of plasma antioxidant activity

The antioxidant activities of plasma samples were analyzed by flow injection analyses coupled to a luminol chemiluminescence (CL; Lumi-Flo, Chrono-Log, USA) assay described by Sariahmetoglu et al. (2003). Briefly, a continuous CL signal from H_2O_2 [10^{-4} M; in the presence of 10^{-4} M luminol and 10^{-5} M Co^{+2} in Hank's buffered salt solution (HBSS) at pH 7.4] was obtained. The H_2O_2-dependent CL signal was inhibited by the plasma samples.

2.9. Drugs and chemicals

Indomethacin (FAKO Ilaclari, Istanbul, Turkey), selective COX-1 inhibitor valeryl salicylate (SPI-Bio, Cedex, France) and selective COX-2 inhibitor SC-58236 (4-(5-(4-chlorophenyl)-3-3-trifloromethyl)-1*H*-pyrazol-1-yl) benzenesulfonamide (Searle, Skokie, IL) were dissolved in 0.5% carboxymethyl cellulose for daily usage and administered 1 h before carrageenan or lipopolysaccharide injection by orogastric route.

For vitamin E supplementation, DL-α-tocopherolhydrogen succinate (Merck, USA) was blended into the powdered food at a concentration of 20 g/kg diet, which equals ~2 g/(kg body wt/day). Vitamin-E-treated groups received powdered food for 3 weeks. Lambda carrageenan, arachidonic acid and lipopolysaccharide (*E. coli* O111:B4, lot no. 2630) were purchased from Sigma–Aldrich Chemie (Taufkirchen, Germany). ^{3}[H]$PGF_{2\alpha}$ was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). All organic solvents that were used for extraction are HPLC grade and were purchased from Merck. The standard, antibody and enzymatic tracer of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 and precoated EIA microtiter plates were purchased from SPI-Bio.

2.10. Statistical analyses

The experimental groups and the control group were compared with Student's *t*-test to compare the formation of

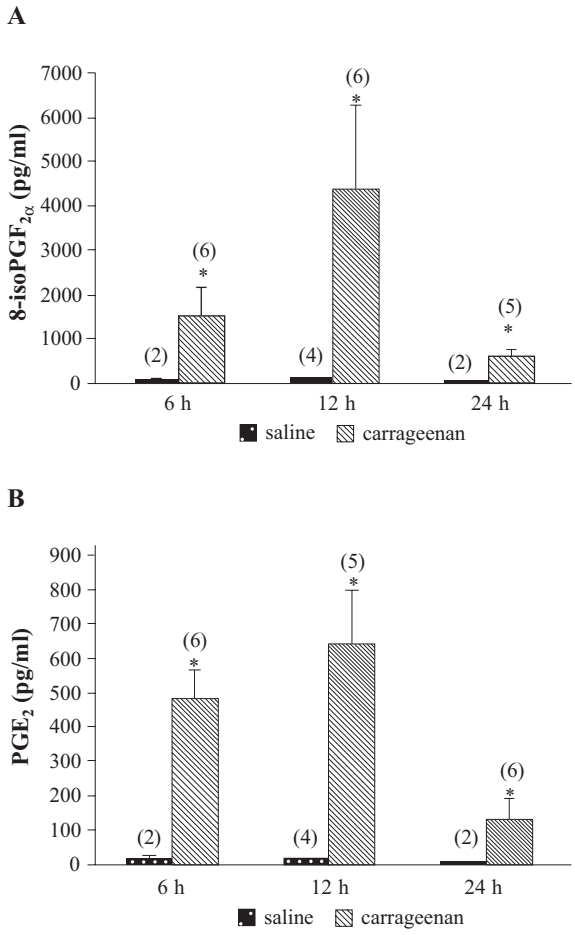

Fig. 1. Time-dependent formation of 8-isoprostaglandin $F_{2\alpha}$ (A) and prostaglandin E_2 (B) in carrageenan-induced air pouch model in rats. Exudate was harvested 6, 12 and 24 h after carrageenan injection. Control experiments were performed by the injection of 2 ml sterile 0.9% saline into the air pouch. Results are expressed as means±S.E.M. The comparison of the carrageenan group with the control group was made by Student's *t*-test. *Significantly different from control group ($P<0.05$). Numbers inside parentheses indicate *n*.

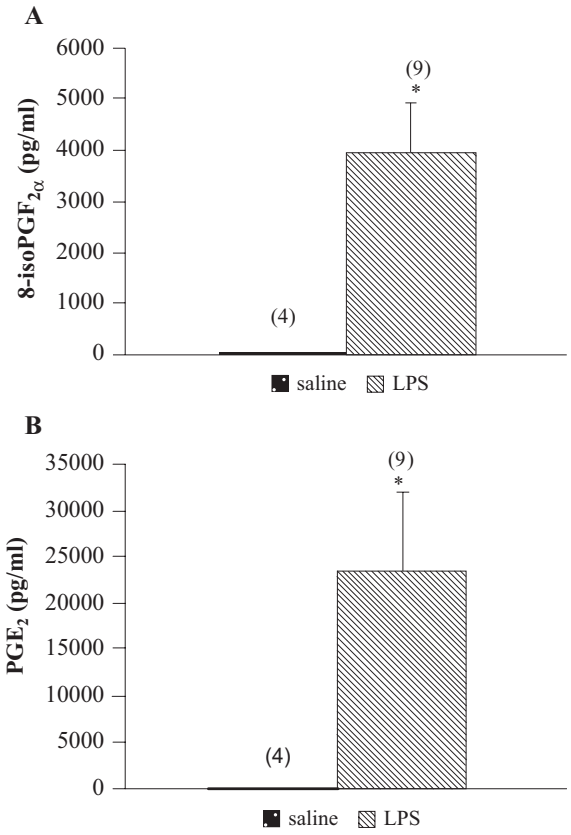

Fig. 2. Formation of 8-isoprostaglandin $F_{2\alpha}$ (A) and prostaglandin E_2 (B) in lipopolysaccharide-induced air pouch model in rats. Results are expressed as means±S.E.M. Comparison of the lipopolysaccharide group with the control group was made by Student's *t*-test. *Significantly different from control group ($P<0.05$). Numbers inside parentheses indicate *n*.

time-dependent 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in experimental inflammation models. Data are presented as mean values±standard error of the mean. The results were accepted statistically significant if $P<0.05$.

Modified Hemm (inhibition) test, which was developed by Schering's Department of Biometrics, was used to evaluate the effect of treatments on eicosanoids formation (Schottelius et al., 2002). To determine the inhibitory effect of cyclooxygenase inhibitors and vitamin E, the difference between the mean values of the carrageenan- and saline-control groups were set to 100%, and inhibition was estimated as:

$$\%\ inhibition = 100 - \left[\left(\left(mean\ value_{\text{treatment group}} - mean\ value_{\text{saline control}}\right) / \left(mean\ value_{\text{carrageenan or lipopolysaccharide group}} - mean\ value_{\text{saline control}}\right)\right) \times 100\right]$$

The inhibition thus determined was tested against 0 (zero) by using Student's *t*-test. $P<0.05$ was considered as significant. Data were presented as mean±standard error of the mean.

3. Results

3.1. Time-dependent formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in carrageenan-induced air pouch model

To investigate the time-dependent production of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in carrageenan-induced air pouch model, exudate was harvested 6, 12 and 24 h after carrageenan injection in different groups. 8-

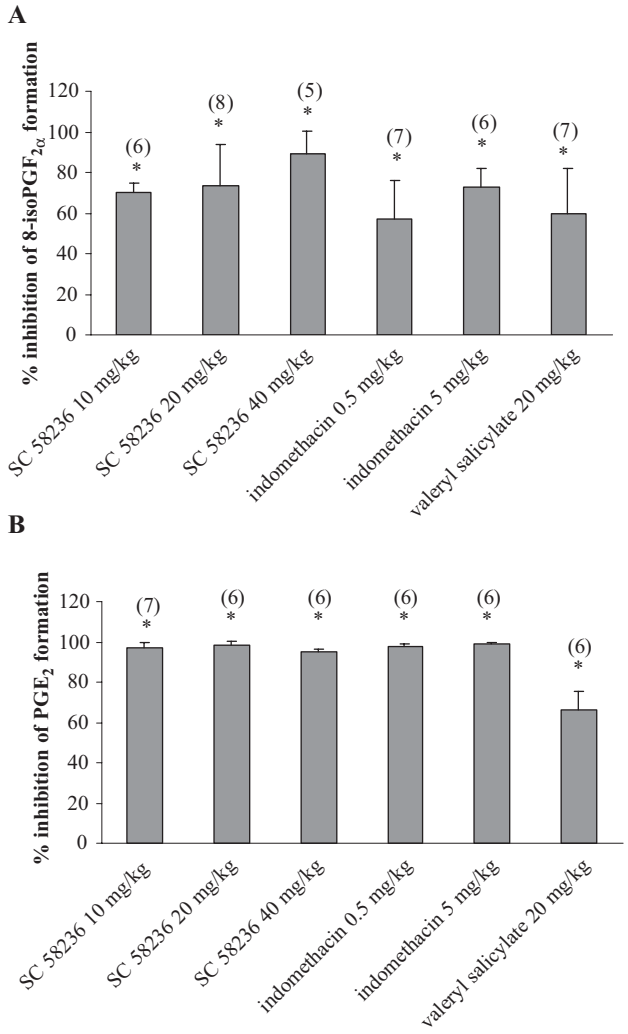

Fig. 3. Effect of indomethacin (0.5 and 5 mg/kg), selective cyclooxygenase-1 inhibitor valeryl salicylate (20 mg/kg) and selective cyclooxygenase-2 inhibitor SC-58236 (10, 20 and 40 mg/kg) on 8-isoprostaglandin $F_{2\alpha}$ (A) and prostaglandin E_2 (B) levels in carrageenan-induced air pouch model in rats. Inhibitors were given 1 h before carrageenan injection by orogastric route. Exudate was harvested 12 h after carrageenan injection. The modified Hemm (inhibition) test was used to evaluate the effect of treatments on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 formation. Details were given in Materials and methods. Whether the inhibition in the treatment group was different from 0 (zero) was evaluated by Student's *t*-test. Results are expressed as percentage of inhibition of formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 and represent means±S.E.M. *Statistically significant ($P<0.05$). Numbers inside parentheses indicate *n*.

isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels reached their maximum values 12 h after carrageenan injection (Fig. 1). Therefore, exudate was harvested 12 h after carrageenan injection in later experiments.

3.2. Formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 in lipopolysaccharide-induced air pouch model

To differentiate cyclooxygenase-2 dependent synthesis of 8-isoprostaglandin $F_{2\alpha}$ from its free-radical catalyzed formation, 8-isoprostaglandin $F_{2\alpha}$ production was assessed in a lipopolysaccharide-induced air pouch model. In this model, it is well known that cyclooxygenase-2 induction occurs without inflammatory reaction. 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels increased significantly compared with the saline control group (Fig. 2). However, the increase in prostaglandin E_2 level was more prominent than the increase in 8-isoprostaglandin $F_{2\alpha}$ level in this model.

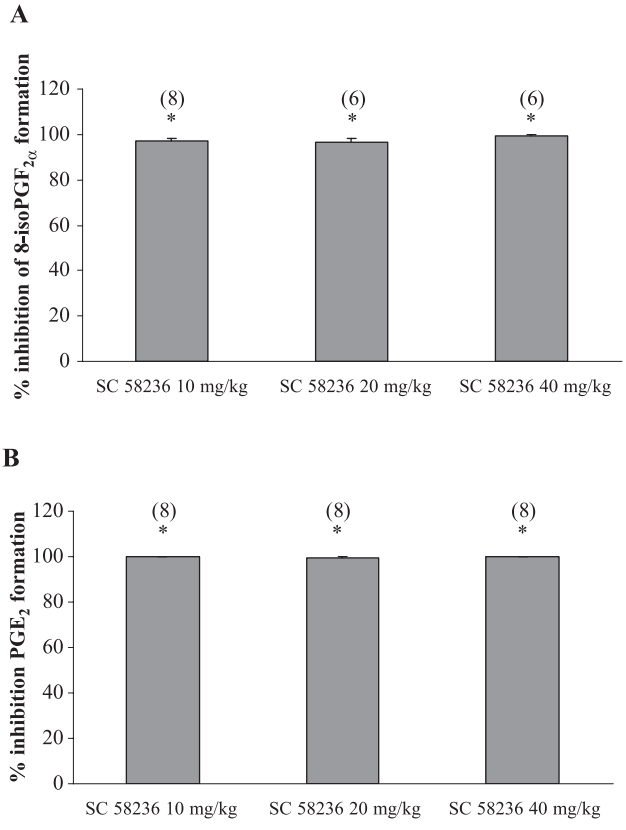

Fig. 4. Effect of selective cyclooxygenase-2 inhibitor SC-58236 (10, 20 and 40 mg/kg) on the 8-isoprostaglandin $F_{2\alpha}$ (A) and prostaglandin E_2 (B) levels in lipopolysaccharide-induced air pouch model in rats. SC-58236 was given 1 h before lipopolysaccharide injection by the orogastric route. Modified Hemm (inhibition) test was used to evaluate the effect of treatments on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 formation. Details were given in Materials and methods. Whether the inhibition in the treatment group was different from 0 (zero) was evaluated by Student's *t*-test. Results are expressed as percentage of inhibition of formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 and represent means±S.E.M. *Statistically significant ($P<0.05$). Numbers inside parentheses indicate *n*.

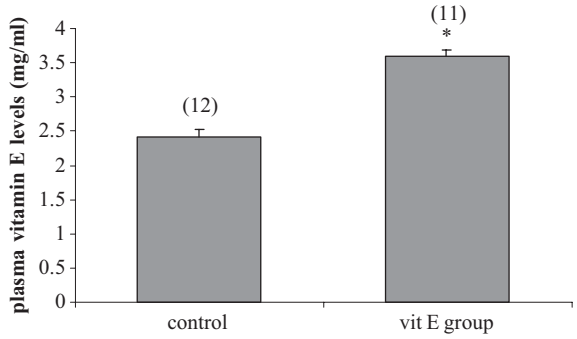

Fig. 5. Effect of supplementation of vitamin E on plasma α-tocopherol concentration. Vitamin E (DL-α-tocopherolhydrogen succinate) was blended into the powdered diet at a concentration of 20 g/kg diet, which equals ~2 g/(kg body wt/day). Vitamin E treated groups received this diet for 3 weeks. The control group received ordinary diet. Plasma α-tocopherol concentration was quantitated by the HPLC method. Details were given in Materials and methods. Results are expressed as means±S.E.M. The comparison of plasma α-tocopherol concentration of vitamin E and control group was made by Student's *t*-test. *Significantly different from control group ($P<0.05$). Numbers inside parentheses indicate *n*.

3.3. Effect of various cyclooxygenase inhibitors on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 level in carrageenan-induced air pouch model

To assess cyclooxygenase-dependent 8-isoprostaglandin $F_{2\alpha}$ formation, nonselective cyclooxygenase inhibitor indomethacin (0.5 and 5 mg/kg), selective cyclooxygenase-1 inhibitor valeryl salicylate (20 mg/kg) and selective cyclooxygenase-2 inhibitor SC-58236 (10, 20 and 40 mg/kg) were used. These inhibitors were given by orogastric route 1 h before carrageenan injection.

All of these inhibitors inhibited both 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 formation significantly (Fig. 3).

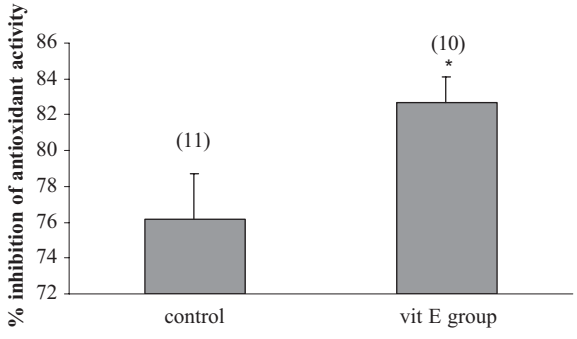

Fig. 6. Effect of supplementation of vitamin E on plasma antioxidant activity. Vitamin E (DL-α-tocopherolhydrogen succinate) was blended into the powdered diet at a concentration of 20 g/kg diet, which equals ~2 g/(kg body wt/day). Vitamin E treated groups received this diet for 3 weeks. The control group received ordinary diet. The antioxidant activities of plasma samples were analyzed by flow injection analysis coupled to luminol chemiluminescence assay. Details were given in Materials and methods. Results are expressed as means±S.E.M. The comparison of plasma antioxidant activity of vitamin E group and control group was made by Student's *t*-test. *Significantly different from the control group ($P<0.05$). Numbers inside parentheses indicate *n*.

3.4. Effect of selective cyclooxygenase-2 inhibitor, SC-58236, on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels in the lipopolysaccharide-induced air pouch model

Three different doses of SC-58236 (10, 20 and 40 mg/kg) inhibited nearly 100% of both 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 productions in this model (Fig. 4).

3.5. Effect of supplementation of vitamin E on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 levels in the carrageenan- and lipopolysaccharide-induced air pouch models

Vitamin E supplementation for 3 weeks increased significantly the plasma α-tocopherol concentration (Fig. 5) and plasma antioxidant capacity in rats (Fig. 6). This supplementation inhibited 8-isoprostaglandin $F_{2\alpha}$ formation both in carrageenan- and lipopolysaccharide-induced air pouch models (Fig. 7), whereas this supplementation inhibited prostaglandin E_2 production only in the lipopolysaccharide-induced air pouch model (Fig. 7).

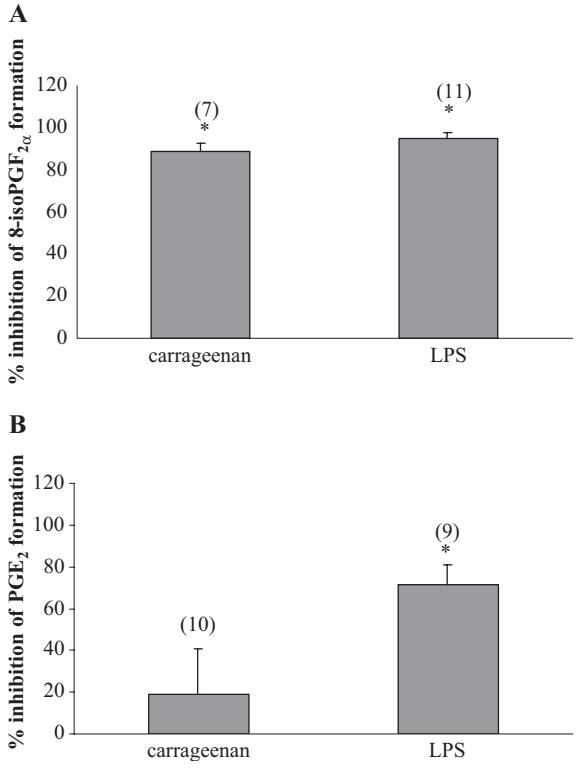

Fig. 7. Effect of supplementation of vitamin E on 8-isoprostaglandin $F_{2\alpha}$ (A) and prostaglandin E_2 (B) levels in carrageenan- and lipopolysaccharide-induced air pouch model in rats. Vitamin E (DL-α-tocopherolhydrogen succinate) was blended into the powdered diet at a concentration of 20 g/kg diet, which equals ~2 g/(kg body wt/day). Vitamin E treated groups received this diet for 3 weeks. The control group received ordinary diet. Modified Hemm (inhibition) test was used to evaluate the effect of treatments on 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 formation. Details were given in Materials and methods. Whether the inhibition in the treatment group was different from 0 (zero) was evaluated by Student's *t*-test. Results are expressed as percentage of inhibition of formation of 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 and represent means±S.E.M. *Statistically significant ($P<0.05$). Numbers inside parentheses indicate *n*.

4. Discussion

Inflammation is a complex process that occurs with the contribution of various mediators to diverse cellular events. Among many others, participation of reactive oxygen species in inflammation is well known. These species are controlled by the defence mechanism of an organism. However, their uncontrolled or prolonged production leads to chronic inflammatory diseases, such as rheumatic disease, pulmonary inflammation and inflammatory bowel disease (Ozaki et al., 1986; Tao et al., 2003; Oldenburg et al., 2001). Although there are many different methods to assess the production of reactive oxygen species, such as thiobarbituric acid reactive substance (TBARs; Longmire et al., 1994), malondialdehyde and lipid hydroperoxides (Lynch et al., 1993; Matthews et al., 1993), their reliability is still controversial. Recently, the quantitation of isoprostanes in urine, plasma and other biological fluids has extensively been used to assess free-radical-induced damage. This method has been accepted as a reliable tool and has been considered superior to other methods, for it can be applied in vivo. The first demonstration of in vivo production of isoprostanes in human has been due to Morrow et al. (1990).

In the present study, the level of 8-isoprostaglandin $F_{2\alpha}$, along with prostaglandin E_2, was found to be increased in exudate in carrageenan-induced air pouch model in rats. It has been shown that the level of isoprostanes elevates in some chronic immunoinflammatory conditions, such as scleroderma, systemic lupus erythematosus, antiphospholipid antibodies syndrome and different rheumatic diseases (Stein et al., 1996; Iuliano et al., 1997; Basu et al., 2001), as well as in certain chronic diseases, such as diabetes mellitus (Gopaul et al., 1995; Davi et al., 1999), hypercholesterolemia (Reilly et al., 1998) and Alzheimer's disease (Pratico and Delanty, 2000). To the best of our knowledge however, the present report is the first demonstration of increased 8-isoprostaglandin $F_{2\alpha}$ in an acute inflammation model.

It is well known that isoprostanes are synthesized by a cyclooxygenase-independent mechanism. However, there are some studies indicating that they may also be produced by a cyclooxygenase-2-dependent mechanism (Pratico and Fitzgerald, 1996). It may be important to differentiate free-radical-catalyzed formation of 8-isoprostaglandin $F_{2\alpha}$, which may take place in inflammatory situations, from the one that depends on cyclooxygenase-2 in the absence of inflammatory reaction. For this purpose, the production of 8-isoprostaglandin $F_{2\alpha}$ was assessed in lipopolysaccharide-induced air pouch model in rats. Unlike the carrageenan-induced air pouch model, this model provides cyclooxygenase-2 induction without inflammatory reaction. Here, 8-isoprostaglandin $F_{2\alpha}$ level was found to be increased in air pouch compared with the control (Fig. 2), which indicates

cyclooxygenase-2-dependent 8-isoprostaglandin $F_{2\alpha}$ syntheses. Levels of 8-isoprostaglandin $F_{2\alpha}$ in the carrageenan- and lipopolysaccharide-induced air pouch models were nearly the same (respectively, 4340.99±1900.39 and 3932.82±831.81 pg/ml), whereas prostaglandin E_2 synthesis was 36.5-fold high in the lipopolysaccharide-induced air pouch model compared with that in the carrageenan-induced air pouch model. The ratio of prostaglandin E_2 to 8-isoprostaglandin $F_{2\alpha}$ in the lipopolysaccharide-induced air pouch model was 1:5.9, consistent with the one that has been reported by Pratico and Fitzgerald (1996) in lipopolysaccharide-induced human monocytes (1:5.3).

Further evidence for the cyclooxygenase-dependent formation of isoprostanes comes from the present observation that cyclooxygenase inhibitors suppressed the formation of 8-isoprostaglandin $F_{2\alpha}$. Nonselective cyclooxygenase inhibitor indomethacin (0.5 and 5 mg/kg), selective cyclooxygenase-1 inhibitor valeryl salicylate (20 mg/kg) and selective cyclooxygenase-2 inhibitor SC-582368 (10, 20 and 40 mg/kg) inhibited both 8-isoprostaglandin $F_{2\alpha}$ and prostaglandin E_2 formation in the carrageenan-induced air pouch model. The most prominent inhibition of 8-isoprostaglandin $F_{2\alpha}$ formation was observed with high doses of a selective cyclooxygenase-2 inhibitor SC-582368 (89.49±10.7% inhibition). The latter fact further supports the idea that the formation of 8-isoprostaglandin $F_{2\alpha}$ is, at least partly, cyclooxygenase-2 dependent. On the other hand, low doses of indomethacin (0.5 mg/kg) also inhibited the formation of 8-isoprostaglandin $F_{2\alpha}$ (57.19±18.81% inhibition). At such a low dose, indomethacin is not expected to inhibit cyclooxygenase-2. Therefore, it seems likely that cyclooxygenase-1 also contributes to the formation of 8-isoprostaglandin $F_{2\alpha}$. The inhibition of 8-isoprostaglandin $F_{2\alpha}$ formation by valeryl salicylate, a selective cyclooxygenase-1 inhibitor (Bhattacharyya et al., 1995) also supports this suggestion. Both indomethacin and SC-582368 caused near-complete inhibition of the formation of prostaglandin E_2. Unexpectedly, valeryl salicylate, a selective cyclooxygenase-1 inhibitor, also inhibited (59.48±22.76% inhibition) of the formation of prostaglandin E_2. However, there are some studies that show the cooperation of cyclooxygenase-1 and cyclooxygenase-2 in inflammation. It was shown that prostaglandin levels in cyclooxygenase-2-deficient mice were similar to that of wild-type and indomethacin, at a dose that suppressed cyclooxygenase-1 activity, and significantly reduced both inflammation and prostaglandin levels at the site of inflammation (Wallace et al., 1998). Langenbach et al. (1995) reported that arachidonic acid-induced ear swelling in cyclooxygenase-1-deficient mice was found low compared with that of wild-type mice.

The inhibitory effect of SC-582368 and indomethacin on the formation of 8-isoprostaglandin $F_{2\alpha}$ are not dose dependent. It is possible to speculate that the above-mentioned effect of these inhibitors on 8-isoprostaglandin $F_{2\alpha}$ formation may be, at least partly, unrelated to the inhibition of cyclooxygenase. There are many cyclooxygenase-independent effects of NSAIDs. Among these effects, the antioxidant properties of this class of drugs are well known. In this context, the antioxidant properties of both nonselective cyclooxygenase inhibitors (Parij and Nève, 1996) and selective cyclooxygenase-2 inhibitors (Van Antwerpen and Nève, 2004) were published. As discussed in a later paragraph, vitamin E, which has also antioxidant properties, inhibited 8-isoprostaglandin $F_{2\alpha}$ formation.

In the present study, we also evaluated the effect of vitamin E supplementation as an antioxidant on the formation of 8-isoprostaglandin $F_{2\alpha}$. It has been reported that the administration of vitamin E decreased lipid peroxidation, augmented the activities of antioxidant enzymes and reduced the content of malondialdehyde in the kidneys of diabetic rats (Kedziora-Kornatowska et al., 2003). Recent studies have shown that immobilization-induced oxidative stress in rats could be reversed by vitamin E administration, which restored the decreased activities of superoxide dismutase, glutathione-*S*-transferase and catalase, and thus decreased the lipid peroxidation (Zaidi and Banu, 2004). Hence, the inhibition of 8-isoprostaglandin $F_{2\alpha}$ formation by vitamin E, as being an effective antioxidant, is not surprising. There are indeed many examples that show such an effect of vitamin E: Vitamin E supplementation significantly reduced high urinary isoprostane levels in non-insulin-dependent diabetes mellitus patients (Davi et al., 1999) or in hypercholesterolemia patients (Davi et al., 1997). Likewise, vitamin E reduced elevated isoprostane levels in cyclosporin-A-induced renal toxicity in rats (Kanji et al., 1999). Parallel to these observations, we also show in the present study that vitamin E inhibits the formation of 8-isoprostaglandin $F_{2\alpha}$ in the carrageenan- and lipopolysaccharide-induced air pouch models (88.81±3.76% and 94.82±2.70% inhibition, respectively), with an increase in plasma antioxidant activity. However, the present observation can hardly be explained solely by direct antioxidant activity that vitamin E imparts on lipid oxidation. As discussed above, the production of 8-isoprostaglandin $F_{2\alpha}$ appears to be cyclooxygenase-2 dependent in the lipopolysaccharide-induced air pouch model and depends partly on cyclooxygenase-2 in the carrageenan-induced air pouch model. One possible explanation may therefore be that vitamin E can affect cyclooxygenase-2 activity directly, or indirectly by its antioxidant property. There are indeed observations supporting such an effect of vitamin E on cyclooxygenase activity: Vitamin E inhibits lipopolysaccharide-induced prostaglandin E_2 synthesis in murine J774.1A macrophage cell line (Abate et al., 2000) or in mouse peritoneal macrophage (Wu et al., 2001); it increases the inhibitory effect of aspirin on prostaglandin E_2 synthesis independently of its antioxidant activity (Abate et al., 2000). Likewise, in the present study, the supplementation of vitamin E inhibited the formation of prostaglandin E_2 in the lipopolysaccharide-induced air pouch model (71.49±8.64% inhibition), but the not in carrageenan-induced air pouch model, in which cyclo-

oxygenase-1 is also expected to be involved (Wallace et al., 1998; Langenbach et al., 1995). The latter point suggests the possibility that the effect of vitamin E on cyclooxygenase activity may be biased towards cyclooxygenase-2.

In conclusion, we showed that 8-isoprostaglandin $F_{2\alpha}$ can be formed by cyclooxygenase-2 in an acute inflammation model (i.e., carrageenan-induced air pouch model) by using several cyclooxygenase inhibitors. The production of 8-isoprostaglandin $F_{2\alpha}$ in the lipopolysaccharide-induced air pouch model provides further evidence for the involvement of cyclooxygenase-2 in 8-isoprostaglandin $F_{2\alpha}$ formation. It may be interesting to investigate cyclooxygenase-independent, free-radical-catalyzed isoprostane formation and assess the effect of isoprostanes on various inflammatory parameters in chronic immunoinflammatory conditions.

Acknowledgements

This study was supported by a grant from Ankara University research fund (2002-08-09-087). We would like to thank Searle for providing us SC-582368. The authors would also like thank Professor Ical Çakici and Dr. Nilüfer Turan for the analyses of antioxidant activity of plasma and Professor Ongun Onaran for a helpful criticism of the manuscript.

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