GREEN TEA EXTRACT SUPPLEMENT INHIBITION OF HMGB1 RELEASE IN RATS EXPOSED TO CIGARETTE SMOKE

Tawee Saiwichai[1], Vanida Sangalangkarn[2], Ko-ichi Kawahara[3], Yoko Oyama[3], Sirintip Chaichalotornkul[4], Somphong Narkpinit[4], Pongchai Harnyuttanakorn[5], Pratap Singhasivanon[6], Ikuro Maruyama[3] and Salunya Tancharoen[2]

[1]Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University; [2]Department of Pharmacology, Faculty of Dentistry, Mahidol University; [4]Department of Pathobiology, Faculty of Science, Mahidol University; [6]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [3]Department of Laboratory and Vascular Medicine, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima, Japan; [5]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Abstract. Tobacco-smoke exposure is linked to carcinogenic, oxidative and inflammatory cellular reactions. Green tea has been reported to have anti-release properties against various pro-inflammatory cytokines. To determine the effects of green tea extract (GTE) on serum high mobility group box-1 (HMGB1) levels in rats exposed to cigarette smoke (CS), we divided rats into 4 treatment groups: (1) CS only, (2) dietary supplement with GTE (3 mg/d) and CS (GCS1), (3) dietary supplement with GTE (4.5 mg/d) and CS (GCS2) and (4) a control group. HMGB1 and cotinine serum levels were analyzed by ELISA. The average serum HMGB1 level in the CS group was significantly higher than the other groups ($p < 0.01$), indicating the release of HMGB1 into the blood was stimulated by CS exposure, while GTE consumption suppressed HMGB1 levels. Rats exposed to CS had an average serum cotinine level of 37 ng/ml, indicating tobacco related compounds were present in the rats' blood. However, treatment with GTE did not reduce cotinine levels in all groups. Cotinine stimulated HMGB1 secretion in a dose- and time-dependent manner, and HMGB1 levels were suppressed by GTE in murine macrophage cell lines. Our results show GTE supplementation may offer beneficial systemic effects and suppress HMGB1 by protecting against cell inflammation.

Key words: green tea, HMGB1, cigarette smoke

INTRODUCTION

Sidestream and mainstream smoke from burning tobacco contains over 4,000 chemicals, including more than 50 known carcinogens (Moir *et al,* 2007). Non-smokers exposed to secondhand smoke also inhale nicotine and other toxic chemicals (Department of Public Health, Country of Los Angeles Public Health, 2009). Plasma levels of nicotine and its residual metabolite, cotinine, correlate significantly with levels of cigarette smoke (CS) exposure (Dziuda and Grzybowski, 1999; Stepanov

Correspondence: Dr Salunya Tancharoen, Department of Pharmacology, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand.
Tel: 66 (0) 2203 6481 ext 18; Fax: 66 (0) 2203 6484
E-mail: dtsta@mahidol.ac.th

et al, 2007). Exposure to secondhand smoke impairs the function of various organs and induces respiratory illness via inflammatory processes and oxidative stress leading to cell injury (Rona *et al,* 1985; Bruske-Hohlfeld, 2009).

HMGB1 is a ubiquitously expressed, highly conserved, chromosomal protein. It stabilizes the nucleosomal structure; thereby, facilitating the binding of specific transcription factors, including steroid hormone receptors (Lotze and Tracey, 2005). Oxidative stress induces the release of HMGB1 from the chromosomes and its subsequent cytoplasmic translocation (Kikuchi *et al,* 2009). Extracellular HMGB1 can bind to cell surface receptors, such as receptors for advanced glycation end products (RAGEs), TLR-2 and -4 and possibly, unknown receptors (Raucci *et al,* 2007). HMGB1 can be secreted by macrophages and monocytes, or released from necrotic cells as a soluble molecule into circulating blood. This release is initiated at tissue injury sites and activates inflammatory responses (Kornblit *et al,* 2008). HMGB1's well-documented pro-inflammatory activity is found in many cell types, including inflammatory cells, endothelial cells, and smooth muscle cells (Neeper *et al,* 1992; Erlandsson and Andersson, 2004). Recent evidence identifies HMGB1 as a cytokine-like mediator of delayed endotoxin lethality and acute lung injury (Wang *et al,* 2001).

Green tea (*Camellia sinensis*) is rich in polyphenolic compounds; its polyphenolic fraction reduces the expression of pro-inflammatory cytokines and acts as a powerful antioxidant against lipid peroxidation in biological systems (Vinson *et al,* 1995). Pre-clinical animal studies suggest green tea has anti-inflammatory activity in patients with arthritis and pulmonary inflammation (Rahman and Kilty, 2006; Miyake *et al,* 2008). A major ingredi-

ent of green tea, epigallocatechin gallate, suppresses TNF-α-induced NF-kappaB activation (Baeuerle and Henkel, 1994), increases nitric oxide synthesis to block endothelial exocytosis, cutting vascular inflammation (Yamakuchi *et al,* 2008), and protects mice against lethal endotoxemia (Li *et al,* 2007).

In this study, we hypothesized green-tea extract, a naturally occurring compound with known anti-inflammatory properties, may have beneficial therapeutic effects against CS, involving significant inflammation and oxidative stress via suppression of HMGB1, a novel pro-inflammatory mediator.

MATERIALS AND METHODS

Animal model and experimental design

Male rats *(Rattus norvegicus)* (14 wks old, weighing 280-300 g) were purchased from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand), kept under pathogen-free conditions on a 12-hour light/dark cycle at a controlled temperature (22ºC), and food and water was provided *ad libitum*. The rats were allowed to acclimatize for 1 week before the start of treatment. All animal procedures were approved by the Animal Care and Use Committee, Mahidol University (No. FTM-ACUC 012/2007). Rats were randomly assigned to four groups (12 rats per group): (1) CS only, (2) dietary supplement with GTE (3 mg/d) and CS (GCS1), (3) dietary supplement with GTE (4.5 mg/d) and CS (GCS2), and (4) the control group in which rats were given neither GTE nor CS. Treatments were randomized, and investigators were blinded to the specific treatment.

Green-tea extraction and catechin analysis

Fresh green tea leaves from West Java, Indonesia were prepared as described pre-

viously (Maiti *et al,* 2003), with slight modification. The total catechin content of the extract was determined by high performance liquid chromatography using a Waters 2996 separation module with a 2996 photodiode array detector. The extract contained 39% (w/w) epigallocatechin-3-gallate (EGCC), 4.5% epigallocatechin (EGC), 17.52% epicatechin gallate (ECG), 3.7% epicatechin (EC), and 1.4% catechin. Standard catechin derivatives were supplied by Sigma Biochemicals (St. Louis, MO).

Rat green tea consumption

The rats were fasted for 2 hours; the experimental groups (GCS1 and GCS2) then received a diet supplemented with GTE at 3 mg/day and 4.5 mg/day, respectively, for the entire 8-week experiment. The control group of rats was given the same diet without GTE. Food consumption and body weight were measured daily and weekly, respectively. When the rats finished the GTE supplemented food, normal food and water were provided *ad libitum* then fasted again prior to being given the GTE supplemented food again.

Smoke exposure protocol

After receiving GTE for 4 weeks, the rats were exposed to the smoke of 4 cigarettes, 5 days/week, twice a day for 4 weeks (weeks 5-8). The smoke was produced by introducing ignited cigarettes into the sterile, ventilated, whole-body exposure chamber (35 x 70 x 40 cm; 4 rats / container). Room temperature was maintained at 22-25ºC and relative humidity at approximately 40%. To facilitate handling rats in the control group, they were placed in a chamber and exposed to laboratory air. To eliminate day-time nicotine absorption bias, the experimental rats were exposed to CS at 8:00 AM and 5:00 PM and left in the chamber for 10 minutes before being returned to their cages.

Cell culture and treatment

Murine macrophage-like RAW 264.7 cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium (GibCo BRL, Grand Island, NY) supplemented with 10% fetal bovine serum and 2 mmol/l glutamine (Hyclone Logan, UT). At 80-90% confluence, the RAW 264.7 cells were washed twice, then cultured in serum-free OPTI-MEM I medium (GibCo BRL) before stimulation with various concentrations (0.1, 1, 10, 100, 1,000 ng/ml) of cotinine (Sigma; St Louis, MO) for 16 hours. The cell cultures were then subjected to the absence or presence of GTE at various concentrations (0.025, 0.25, 2.5, and 25 µg/ml) for 1 hour and were stimulated with 0.5 µg/ml lipopolysaccharide (LPS) in the culture medium, while the negative cells had polymyxin B add at 6 units per pg of LPS.

Cell viability assay

Cell viability was assessed by a modified methyl thiazolyl-diphenyl-tetrazolium bromide (MTT) assay (Kawahara *et al,* 2008). Briefly, cells were exposed to various concentrations of cotinine (0.1, 1, 10, and 100 ng/ml). After 24 hours, MTT solution was added to each 6-well plate. After another 3 hours, dimethyl sulfoxide (DMSO) was added; the plates were then incubated at 37ºC for 24 hours. Absorbance was read at 570 nm wavelength with an automatic microtiter plate reader (ImmunoMini NJ-2300; InterMed, Tokyo, Japan).

Determination of HMGB1 levels in rat serum and cell culture supernatant

The rat blood was collected in blood-collection tubes (Terumo, Tokyo, Japan), then the serum was obtained and stored at -80ºC until use. HMGB1 levels in serum and cell-culture supernatants were as-

sessed by ELISA (Shino-Test Corporation, Kanagawa, Japan); the absorbance was read at 450 nm. Each sample evaluation was duplicated and compared with a standard curve.

Determination of serum cotinine levels

Smoke inhalation was monitored by measuring blood cotinine levels (Hapidin *et al,* 2007). Serum cotinine was measured by ELISA according to the manufacturer's protocol (DRG Instruments, Marburg, Germany). Briefly, a 10 µl aliquot of diluted serum was incubated with a 100 µl dilution of a horseradish peroxidase enzyme-labeled cotinine derivative in a 96-well microplate coated with fixed amounts of high-affinity purified polyclonal cotinine antibody. The wells were washed thoroughly and a chromogenic substrate was added. Color production was stopped using a dilute acid-stop solution and the wells were read at 450 nm. The minimum detectable concentration was 1 ng/ml. All experiments were performed in duplicate.

Statistical analysis

Serum HMGB1 levels in each experimental group were compared by analysis of variance (ANOVA) with repeated measurement. The results of the densitometric analysis of protein signal differences were assessed using the Student's *t*-test. Significant differences in inhibition-study tests were analyzed by the Dunnett's multiple comparison procedure. The results were expressed as means ± SD; a *p*-value ≥ 0.05 was considered significant.

RESULTS

GTE effect on CS-induced HMGB1 and cotinine level

After 4 weeks of GTE supplementation, the sera were collected every 2 weeks. HMGB1 levels were not different between
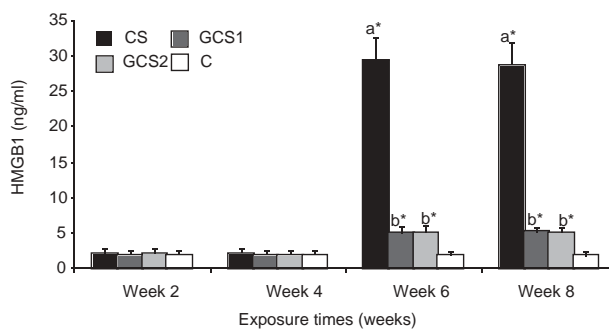
a* = Significant differences found among different groups during the study period
b* = Significant differences found between the CS and C groups during the study period

Fig 1–GTE attenuation of HMGB1 in rat serum. CS-rats exposed to cigarette smoke but given no GTE; GCS1-rats exposed to cigarette smoke and given a diet supplemented with GTE at 3 mg/d; GCS2-rats exposed to cigarette smoke and given a diet supplemented with GTE at 4.5 mg/d (GCS2); C-control rats not exposed to cigarette smoke or given GTE. The results are given as mean ± SD; *n* = 12 rats per group.

the experimental groups at weeks 2 and 4. Treatment with 3 mg/d or 4.5 mg/d GTE prior to CS exposure (weeks 5-8) led to significantly decreased HMGB1 concentrations ($p < 0.01$; Fig 1). The HMGB1 levels detected at weeks 6 and 8 of the experiment were CS: 29.37 ± 6.72, GCS1: 5.08 ± 1.09, GCS2: 4.97 ± 1.11, C: 1.91 ± 0.87 and CS: 28.74 ± 6.99, GCS1: 5.20 ± 1.02, GCS2: 4.88 ± 0.84 and C: 1.84 ± 0.72 ng/ml, respectively.

No significant differences were found in the body weight of rats among the CS exposure groups (CS, GCS1, and GCS2); however, the average body weight of the rats exposed to CS was significantly lower than the controls (Table 1). To evaluate for

Table 1
Comparison of rat body weights among study groups.

	Body weight of rats (g)			
	CS ($n = 12$)	GCS1 ($n = 12$)	GCS2 ($n = 12$)	C ($n = 12$)
Week 0	291 ± 6.96	291 ± 5.94	289 ± 7.66	288 ± 44.13
Week 8	370 ± 7.05	382 ± 20.32	365 ± 17.38	411 ± 22.72*

* $p = 0.05$

kidney toxicity due to consumption of GTE and exposure to CS, blood urea nitrogen (BUN) levels were measured by Spotchem (Arkray, Kyoto, Japan); these were not significantly different among the groups: CS: 18.45 ± 1.40; GCS1: 17.81 ± 0.56; GCS2: 16.22 ± 0.39; and C: 15.32 ± 0.12 mg/dl.

After CS exposure for 4 weeks, the CS-exposed rats (CS, GCS1 and GCS2 groups) had significantly higher serum cotinine levels than the C-group rats [37.2, 34.0, and 31.43, respectively, *vs* undetectable (< 0.05 ng/ml) $p < 0.05$] (Fig 2), indicating the presence of tobacco-related compounds in the blood of the CS-exposed rats. However, no significant differences in cotinine levels were observed among the CS, GCS1, and GCS2 groups (Fig 2).

GTE effect on HMGB1 release in cotinine-induced RAW264.7 cells

HMGB1 is released from murine macrophage-like cells, which are commonly used to investigate anti-inflammatory responses (Wang *et al,* 1999). Cotinine was chosen as a marker for tobacco-smoke inhalation, because of its specificity as a nicotine metabolite and its long half-life of 20 hours (Jarvis, 1987). We determined whether cotinine could induce HMGB1 release from RAW264.7 cells by stimulation with cotinine in concentrations similar to those observed in the sera of CS-exposed rats (Fig 2). The cells were stimu-
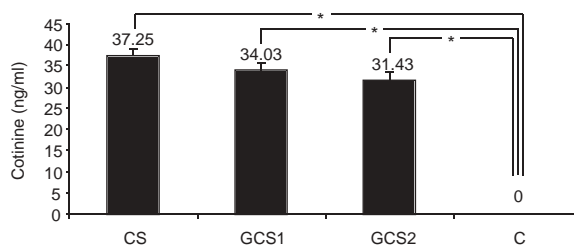

Fig 2–Cotinine levels in CS-exposed rats. *p < 0.05 *vs* control.

lated with 10, 100, and 1,000 ng/ml of cotinine in culture medium; HMGB1 increased significantly in the exposed group. One nanogram per milliliter of cotinine increased HMGB1 to 40 ng/ml, significantly greater than the control ($p < 0.05$); Fig 3A.

To determine if GTE merely shifted the kinetics of cotinine-induced HMGB1 release, cells were pretreated with GTE and then stimulated with cotinine. GTE concentrations of 2.5 and 25 μg/ml significantly suppressed cotinine-induced HMGB1 release compared with cotinine-stimulated cells without GTE supplement (15, 23 and 85 ng/ml; *p*< 0.05; Fig 3B). Polymyxin B (PB) neutralizes LPS biological activity, which induces HMGB1 release in RAW264.7 cells. As a negative control for the specificity of the effects of GTE on HMGB1 under our experimental conditions, we stimulated cells with LPS in the presence or absence of polymyxin B. Our

A

B
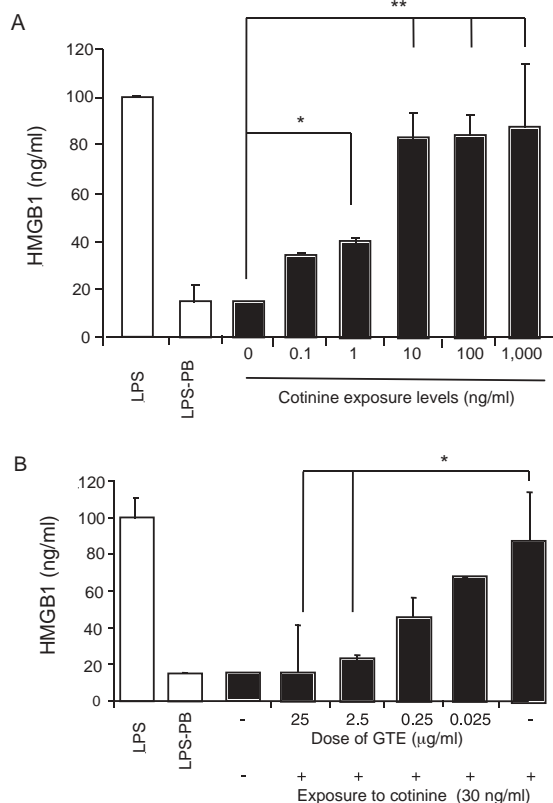

Fig 3–GTE effect on cotinine-induced HMGB1 in RAW264.7 cell culture. A: dose-dependent effect of cotinine; B: dose-dependent effect of GTE on HMGB1 levels in cells exposed to 30 ng/ml cotinine. LPS-cell exposed to lipopolysaccharide (positive control); LPS-PB-cell exposed to lipopolysaccharide and polymyxin B (negative control). $*p < 0.05$, $**p < 0.01$.

data confirm the suppressive effect of GTE on cotinine in cell culture.

Necrotic cells passively release HMGB1 (Scaffidi *et al,* 2002). The presence of 1 µg/ml cotinine significantly stimulated HMGB1 release, but did not decrease cell viability (Fig 4). The GTE concentrations used in our study, even at concentrations that almost completely abrogated HMGB1 release, exhibited no cytotoxicity to the cell
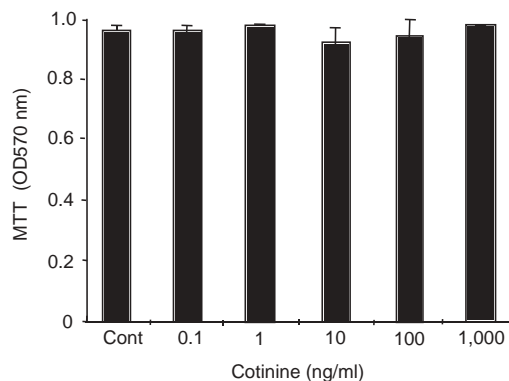

Fig 4–RAW264.7 cell viability as measured by MTT assay following cotinine treatment.

culture, as assessed by propidium iodide uptake (data not shown), demonstrating GTE inhibited HMGB1 release without interfering with cell viability.

DISCUSSION

This study describes for the first time a molecular mechanism behind the association between CS, cotinine, and a newly described cytokine, HMGB1. Green tea compound, with known anti-inflammatory and anti-oxidative properties, suppresses HMGB1 release in response to CS exposure in a rat model and murine macrophage cell lines. These findings suggest CS has an important role in chronic inflammation; GTE may possess an anti-inflammatory effect in rats exposed to CS as shown by HMGB1 levels.

In this study, one cigarette contained $1.1 ± 0.3$ mg nicotine according to analysis by the Research and Development Department, Thailand Tobacco Monopoly. The 8 cigarettes per day (CPD) used in this study contained 8 mg nicotine, equal to 6 mg/kg/d nicotine intake per rat, conferring a total nicotine dose of 25 ng/ml plasma nicotine (Navarro *et al,* 2003), a similar dose to those found in smokers (Trauth *et al,* 1999),

which reportedly induces an inflammatory reaction (Hioki *et al,* 2001). Rats exposed to CS showed significantly elevated serum cotinine levels, peaking at 31.42 ng/ml. This finding suggests CS causes HMGB1 production, reflecting inflammation.

After exposure to 8 CPD for 4 weeks, the rats' weights decreased, which may have been caused by nicotine's ability to suppress appetite and decrease food intake (Jo *et al,* 2002). Green tea consumption is also involved in processes leading to weight loss in rats with diet-induced obesity (Du *et al,* 2005). However, we found no significant weight loss in rats treated with GTE compared to the control group, indicating the GTE used in this study had no significant effect on rat appetite.

Nicotine is quickly converted into its major metabolite, cotinine, commonly used as a biochemical marker of tobacco use (Dziuda *et al,* 1999). The highest median cotinine concentrations were found in the group that reported smoking 10-19 CPD (de Weerd *et al,* 2002). The current study found serum cotinine levels induced by 8 CPD were lower than previous reports (Galeazzi *et al,* 1999; Chalmers *et al,* 2002). Serum cotinine content may vary drastically in smoking patients and by experimental conditions. These differences may be due to variation in cigarette brands and smoking patterns. Our study showed no significant reduction in serum cotinine levels in the GTE-consuming rats, compared with those exposed only to CS. This result was unexpected but consistent with a report showing the elimination half-life of cotinine is longer (50.2 ± 4.7 minutes) than nicotine (8.6 ± 0.4 minutes) in DBA/2 mice (Siu and Tyndale, 2007), suggesting GTE's anti-inflammatory effects are caused by a decreased cytokine release, rather than cotinine production. Another

possible explanation is nicotine is also present in tea leaves, and the nicotine content of tea leaves has been found to be highly variable (Siegmund *et al,* 1999).

Until now, research has focused mainly on determining nicotine's action (Hecht *et al,* 1978). Cotinine up-regulates vascular endothelial growth factor expression in endothelial cells (Conklin *et al,* 2002). Macrophages move through the basement membrane of the vascular endothelium to the site of tissue inflammation (Janeway *et al,* 2005). From this point of view, studies of correlations between cotinine and a vascular pro-inflammatory mediator appear justified. Our *in vitro* studies demonstrate cotinine induces abundant HMGB1 release. In contrast, GTE attenuates cotinine-induced HMGB1 release, but does not reduce serum cotinine levels in CS-exposed rats. The results of this study suggest GTE only suppresses cotinine's role in HMGB1 induction via signaling mechanisms, such as antioxidation (Li *et al,* 2007), but does not act directly as a cotinine antagonist.

In conclusion, the release of HMGB1 and cotinine in rat serum due to CS may be important in the pathophysiology of systemic diseases; modulation of this pathway by GTE could result in new treatment or prevention modalities.

ACKNOWLEDGEMENTS

REFERENCES

Baeuerle PA, Henkel T. Function and activation of NF-kappaB in the immune system. *Annu Rev Immunol* 1994; 12: 141-79.

Bruske-Hohlfeld I. Environmental and occupational risk factors for lung cancer. *Methods Mol Biol* 2009; 472: 3-23.

Chalmers GW, Macleod KJ, Little SA, Thomson LJ, McSharry CP, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. *Thorax* 2002; 57: 226-30.

Conklin BS, Zhao W, Zhong DS, Chen C. Nicotine and cotinine up-regulate vascular endothelial growth factor expression in endothelial cells. *Am J Pathol* 2002; 160: 413-8.

de Weerd S, Thomas CM, Kuster JE, Cikot RJ, Steegers EA. Variation of serum and urine cotinine in passive and active smokers and applicability in preconceptional smoking cessation counseling. *Environ Res* 2002; 90: 119-24.

Department of Public Health (DPH), Country of Los Angeles Public Health. Secondhand smoke. Los Angeles: DHP, 2009. (Cited 2009 Mar 1). Available from: URL: http://www.publichealth.lacounty.gov/hea/docs/Fact_Sheets/Secondhand%20Smoke_Publisher.pdf

Du YT, Wang X, Wu XD, Tian WX. Keemun black tea extract contains potent fatty acid synthase inhibitors and reduces food intake and body weight of rats via oral administration. *J Enzyme Inhib Med Chem* 2005; 20: 349-56.

Dziuda MR, Grzybowski A. Cotinine-the biomarker of exposure to tobacco smoke. *Przegl Lek* 1999; 56: 161-3.

Erlandsson HH, Andersson U. Mini-review: The nuclear protein HMGB1 as a proinflammatory mediator. *Eur J Immunol* 2004; 34: 1503-12.

Galeazzi F, Blennerhassett PA, Qiu B, O'Byrne PM, Collins SM. Cigarette smoke aggravates experimental colitis in rats. *Gastroenterology* 1999; 117: 877-83.

Hapidin H, Othman F, Soelaiman I-N, Shuid AN, Luke DA, Mohamed N. Negative effects of nicotine on bone-resorbing cytokines and bone histomorphometric parameters in male rats. *J Bone Miner Metab* 2007; 25: 93-8.

Hecht SS, Chen CB, Ornaf RM, Hoffmann D, Tso TC. Chemical studies on tobacco smoke LVI. Tobacco specific nitrosamines: origins, carcinogenicity and metabolism. *IARC Sci Publ* 1978; 19: 395-413.

Hioki H, Aoki N, Kawano K, *et al.* Acute effects of cigarette smoking on platelet-dependent thrombin generation. *Eur Heart J* 2001; 22: 56-61.

Janeway CA, Travers P, Walport M, Schlomchik MJ. Immunobiology. New York: Garland Science, 2005: 178-81.

Jarvis MJ. Uptake of environmental tobacco smoke. *IARC Sci Publ* 1987; 81: 43-58.

Jo YH, Talmage DA, Role LW. Nicotinic receptor-mediated effects on appetite and food intake. *J Neurobiol* 2002; 53: 618-32.

Kawahara K, Hashiguchi T, Kikuchi K, *et al.* Induction of high mobility group box 1 release from serotonin-stimulated human umbilical vein endothelial cells. *Int J Mol Med* 2008; 22: 639-44.

Kikuchi K, Kawahara K, Tancharoen S, *et al.* The free radical scavenger edaravone rescues rats from cerebral infarction by attenuating the release of high-mobility group box-1 in neuronal cells. *J Pharmacol Exp Ther* 2009; 329: 865-74.

Kornblit B, Munthe-Fog L, Madsen H, Strom J, Vindelov L, Garred P. Association of HMGB1 polymorphisms with outcome in patients with systemic inflammatory response syndrome. *Crit Care* 2008;12: R83.

Li W, Ashok M, Li J, Yang H, Sama AE, Wang H. A major ingredient of green tea rescues mice from lethal sepsis partly by inhibiting HMGB1. *PLoS ONE* 2007; 2: e1153.

Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in

the immune arsenal. *Nat Rev Immunol* 2005; 5: 331-42.

Maiti TK, Chatterjee J, Dasgupta S. Effect of green tea polyphenols on angiogenesis induced by an angiogenin-like protein. *Biochem Biophys Res Commun* 2003; 308: 64-7.

Miyake M, Ide K, Sasaki K, Matsukura Y, Shijima K, Fujiwara D. Oral administration of highly oligomeric procyanidins of Jatoba reduces the severity of collagen-induced arthritis. *Biosci Biotechnol Biochem* 2008; 72: 1781-8.

Moir D, Rickert WS, Levasseur G, *et al.* A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chem Res Toxicol* 2007; 21: 494-502.

Navarro HA, Basta PV, Seidler FJ, Slotkin TA. Short-term adolescent nicotine exposure in rats elicits immediate and delayed deficits in T-lymphocyte function: critical periods, patterns of exposure, dose thresholds. *Nicotine Tob Res* 2003; 5: 859-68.

Neeper M, Schmidt AM, Brett J, *et al.* Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 1992; 267: 4998-5004.

Rahman I, Kilty I. Antioxidant therapeutic targets in COPD. *Curr Drug Targets* 2006; 7: 707-20.

Raucci A, Palumbo R, Bianchi ME. HMGB1: a signal of necrosis. *Autoimmunity* 2007; 40: 285-9.

Rona RJ, Chinn S, Florey CDV. Exposure to cigarette smoking and children's growth. *Int J Epidemiol* 1985; 14: 402-9.

Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002; 418: 191-5.

Siegmund B, Leitner E, Pfannhauser W. Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. *J Agric Food Chem* 1999; 47: 3113-20.

Siu EC, Tyndale RF. Characterization and comparison of nicotine and cotinine metabolism *in vitro* and *in vivo* in DBA/2 and C57BL/6 mice. *Mol Pharmacol* 2007; 71: 826-34.

Stepanov I, Hecht SS, Lindgren B, Jacob P III, Wilson M, Benowitz NL. Relationship of human toenail nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol to levels of these biomarkers in plasma and urine. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1382-6.

Trauth JA, Seidler FJ, McCook EC, Slotkin TA. Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions. *Brain Res* 1999; 851: 9-19.

Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J Agric Food Chem* 1995; 43: 2800-2.

Wang H, Bloom O, Zhang M, *et al.* HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999; 285: 248-51.

Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. *Am J Respir Crit Care Med* 2001; 164: 1768-73.

Yamakuchi M, Bao C, Ferlito M, Lowenstein CJ. Epigallocatechin gallate inhibits endothelial exocytosis. *Biol Chem* 2008; 389: 935-41.