in vivo *22*: 13-20 (2008)

Suppressive Activity of Epinastine Hydrochloride on Eosinophil Activation *In Vitro*

YU-ICHIRO MOCHIZUKI[1], ATSUKO FURUTA[1], AYAKO FURUYA[1], KEN-ICHI KANAI[1], KAZUHITO ASANO[2] and HARUMI SUZAKI[1]

[1]*Department of Otolaryngology, School of Medicine, Showa University, Tokyo;*
[2]*Division of Physiology, School of Nursing and Rehabilitation Sciences, Showa University, Yokohama, Japan*

Abstract. *The influence of a histamine H_1 receptor antagonist, epinastine hydrochloride (EP), on eosinophil functions was examined in vitro and in vivo. The first set of experiments was undertaken to examine whether EP could suppress eosinophilia and IgE hyperproduction induced by Mesocestoides cortii infection in BALB/c mice. The number of peripheral blood eosinophils and levels of IgE were examined 21 days after infection. Oral administration of EP at a daily dose of 0.3 mg/kg, which is the recommended human therapeutic dose, for 21 days was not able to suppress either peripheral blood eosinophilia or IgE hyperproduction, which was observed in mice infected with M. cortii. The second part of the experiment was designed to examine the influence of EP on eosinophil activation induced by stem cell factor (SCF) stimulation in vitro. Eosinophils were obtained from M. cortii-infected mice and stimulated with SCF in the presence of different concentrations of EP for 24 h. The addition of EP into cell cultures suppressed eosinophil activation induced by SCF stimulation as assessed by measuring the contents of acronym for Regulated upon Activation, Normal T cell Expressed and presumably Secreted (RANTES), macrophage inflammatory protein-1beta (MIP-1β) and leukotriene C_4 (LTC_4) levels in culture supernatants. The minimum concentration of EP which caused significant suppression of factor productions was 25 ng/ml, which is similar to the concentration in plasma after oral administration of the therapeutic dose in humans. These results may suggest that EP exerts inhibitory effects on eosinophil activation and results in favorable modification of the clinical status of allergic patients.*

Correspondence to: Prof. Harumi Suzaki, MD, Department of Otorhinolaryngology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. Tel: +81337848676, Fax: +81337843757, e-mail: h-suzaki@med.showa-u.ac.jp

Key Words: Epinastine, eosinophil, activation, suppression.

Airway mucosal eosinophilia is a prominent feature of allergic airway diseases, such as asthma and rhinitis (1). Eosinophils are believed to play an essential role as final effector cells in the development and maintenance of allergic diseases through the secretion of cationic proteins, lipid mediators, cytokines, and chemokines that can directly cause tissue damage and lead to the exacerbation of inflammatory responses (2). This was confirmed by ultrastructural studies which showed extensive degranulation of eosinophils in airway tissues during active diseases (3, 4). Accordingly, the immunohistochemical analysis revealed high levels of extracellular deposition of granule proteins in the diseased tissues (5). These reports may suggest that the manipulation of eosinophil functions, such as activation and mediator release, will be a therapeutic target in the treatment and prevention of allergic diseases.

Antiallergic agents, such as sodium cromoglycate, fexofenadine and tranilast, have been used in the treatment of allergic disorders for many years, and successful results have been reported (6, 7). These agents are thought to act, at least in part, by stabilizing the cell membrane of effector cells (especially mast cells) and by preventing the release of chemical mediators (7). However, the influence of antiallergic agents on eosinophil functions is poorly understood.

Infection with parasites, especially larval worms, is well-known to induce IgE hyperproduction and peripheral blood eosinophilia in mammalian hosts, including humans and rodents (7, 8). Because these immune responses are quite similar to those in allergic diseases (7, 8), the parasite/host system may be considered to provide a suitable model to examine the therapeutic mechanisms of antiallergic agents on allergic diseases, especially IgE hyperproduction and eosinophilia. *Mesocestoides cortii* is a common parasite in dogs and humans in North and Central America. The larval worm of this parasite, the tetrathyridial larva, has been observed in the peritoneal cavity of wild rodents. Tetrathyridial larva infection in rodents is known to produce peripheral blood eosinophilia and IgE hyperproduction (7).

In the present study, therefore, we have used the *M. cortii*-infected mouse system to examine the influence of epinastine hydrochloride (EP), the most notable histamine H_1 receptor antagonist in Japan (so-called antihistamine), on peripheral blood eosinophilia *in vivo*. We also examined the influence of EP on the activation of eosinophils from mice infected with *M. cortii* by an *in vitro* cell culture technique.

Materials and Methods

Mice. Specific pathogen free male *BALB/c* mice were purchased from Charles River Japan Inc. (Atsugi, Japan). They were maintained in our animal facilities under a controlled environment (25±3˚C, 55±5% humidity and a 12 h light/dark cycle). All animal experimental procedures were approved by the Animal Care and Use Committee of Showa University and were carried out in accordance with the guidelines of the Physiological Society of Japan (9).

Agent. EP was kindly donated from Nihon-Boehringer Ingelheim Co. Ltd. (Tokyo, Japan) as a preservative-free pure powder. This was dissolved in normal saline at a concentration of 100 μg/ml. The mice were given various doses of the agent once a day for 3 weeks *via* a stomach tube at a daily dose of either 100, 150, 200, 300, or 400 μg/kg in a volume not exceeding 0.25 ml.

Parasitological technique. M. cortii, kindly donated by Dr. A. Niwa (School of Medicine, Kinki University, Osaka, Japan), was maintained in mice by intraperitoneal injection of 0.1 ml packed tetrathyridia according to the method described elsewhere (7).

Assay for IgE. Blood was obtained from the retroorbital plexus of mice in a volume of 100 μl on days 0, 4, 7, 14 and 21 relative to the infection. After clotting, the serum was obtained and the total IgE levels were assayed using mouse IgE ELISA test kits (Yamasa Co. Ltd., Chiba, Japan). The ELISA was carried out in duplicate according to the manufacturer's recommendations.

Counting for peripheral blood leukocytes and eosinophils. Total leukocytes in peripheral blood obtained from retroorbital plexus were counted with Turk's staining solution and haemocytometers. The number of eosinophils in peripheral blood was also examined according to the method described previously (10). Briefly, 5 μl of the blood taken from retroorbital plexus was mixed with 20 μl of Hinkelman's solution (Muto Pure Chemicals Co. Ltd., Tokyo, Japan). Eosinophils were counted using haemocytometers.

Preparation of eosinophils. Mice were killed by ether anesthesia 21 days after the injection with 0.1 ml packed tetrathyridia. Peritoneal exudate cells were obtained by washing the mouse peritoneal cavity with 10 ml sterile phosphate-buffered saline (PBS). The cells were washed 3 times with RPMI-1640 medium (SIGMA Co., Ltd., St Louise, MO, USA) supplemented with 10% inactivated fetal calf serum (RPMI-FCS; Nihon Bio-Supply Center, Tokyo, Japan), and incubated in plastic tissue culture plates to remove plastic adherent cells in a humidified atmosphere with 5% CO_2 at 37˚C. After two hours, non-adherent cells (eosinophils) were collected and suspended in RPMI-FCS at a concentration of $5x10^5$ cells/ml. Eosinophils (1.0 ml) were then treated with different doses (10, 20, 25 and 50 ng/ml) of EP for one hour and stimulated with 200.0 ng/ml stem cell factor (R & D Corp., Minneapolis, MN, USA) for 24 h in a final volume of 2.0 ml. The culture supernatants were collected after pelleting cells by centrifugation at 3000 rpm for 15 min at 25˚C and stored at –40˚C until used. The purity of eosinophils was >95% as judged after examination of cells after Giemsa stain.

Assay for eosinophil-derived factors. Levels of eosinophil-derived factors, leukotriene C_4 (LTC_4), acronym for Regulated upon Activation, Normal T cell Expressed and presumably Secreted (RANTES) and macrophage inflammatory protein-1beta (MIP-1β), in culture supernatants were examined using commercially available ELISA test kits (R&D Corp.). The ELISA was carried out duplicate according to the manufacturer's recommendation.

Statistical analysis. Data were analyzed with analysis of variance (ANOVA) followed by Fisher's PLSD test. $P<0.05$ was considered significant.

Results

Influence of M. cortii infection on eosinophilia and IgE production. The first set of experiments was undertaken to examine whether *M. cortii* infection could cause the changes in both the number of eosinophils and IgE levels in peripheral blood. As shown in Figure 1a, the infection of mice with *M. cortii* caused a gradual increase in the number of total leukocytes, which was peaked on day 21. The number of eosinophils in peripheral blood was also increased by *M. cortii* infection (Figure 1b). We then examined the influence of *M. cortii* infection on IgE production. The data in Figure 2 clearly showed that the amount of IgE in peripheral blood was increased by *M. cortii* infection and those from day 14 of infection onward were more than 30-fold that observed in non-infected control.

Effect of EP on eosinophila and IgE production induced by M. cortii infection. The second set of experiments was carried out to examine the influence of EP on eosinophilia and IgE production. As shown in Figure 3a, EP did not suppress the increase in the number of eosinophils induced by *M. cortii* infection: the number of eosinophils in mice treated with 400 μg/kg of EP, which is three times the recommended therapeutic dose for allergic diseases, was nearly identical to that observed in non-treated control. We then examined the influence of EP on IgE production caused by *M. cortii* infection. The data in Figure 3b clearly showed the absence of suppressive effects of EP on IgE hyperproduction as in the case of peripheral blood eosinophilia.

Influence of EP on eosinophil activation in vitro. The third set of experiments was designed to examine whether EP could suppress eosinophil activation in response to immunological stimuli. As shown in Figure 4, the stimulation of eosinophils with SCF at more than 2.5 ng/ml caused significant increase in both RANTES and MIP-1β levels in culture supernatants as

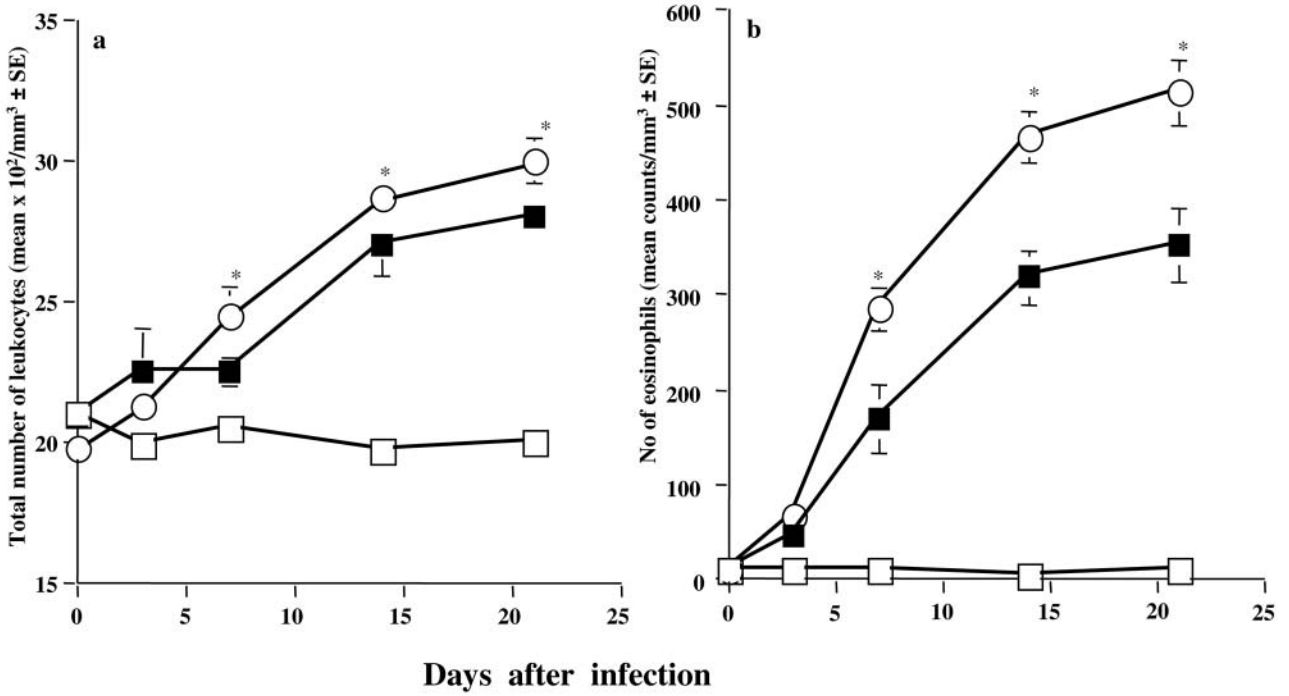

Figure 1. *Influence of Mesocestoides cortii infection on the number of both peripheral blood leukocytes and eosinophils in mice. BALB/c mice were injected intraperitoneally with various numbers of M. cortii larvae on day 0. (a) The number of leukocytes and (b) eosinophils were examined 4, 7, 14 and 21 days after infection. Open square: non-infected; filled square: infected with 100 larvae; open circle: infected with 500 larvae. *Significant ($p<0.05$) as compared to 100 infection.*

compared with non-stimulated control. On the other hand, stimulation of eosinophils with SCF caused a dose-dependent increase in LTC_4 levels in culture supernatants: as the concentration of SCF used for stimulation was increased, the levels of LTC_4 increased, and peaked when the cells were stimulated with SCF at more than 100 ng/ml (Figure 4). We then examined the time course of factor production by eosinophils in response to SCF stimulation. Eosinophils were stimulated with 100 ng/ml SCF and RANTES, LTC_4 and MIP-1β levels were examined 4, 6, 12 and 24 h after stimulation. As shown in Figure 5, the levels of both RANTES and MIP-1β gradually increased and peaked 24 h after SCF stimulation. However, culture supernatants collected 4 h after stimulation contained much higher levels of LTC_4, and these levels were similar (not significant) to those in 24 h culture supernatants (Figure 5). We finally examined the influence of EP on factor production from eosinophils after SCF stimulation. Eosinophils were pre-treated with various doses of EP for 1 h and then stimulated with 100 ng/ml SCF. After 24 h, culture supernatants were collected and RANTES, LTC_4 and MIP-1β levels were examined. EP could dose-dependently inhibit the ability of eosinophils to produce RANTES, LTC_4 and MIP-1β, which was enhanced by SCF stimulation (Figure 6). The minimum concentration of EP which caused significant suppression of factor production was 25.0 ng/ml (Figure 6).

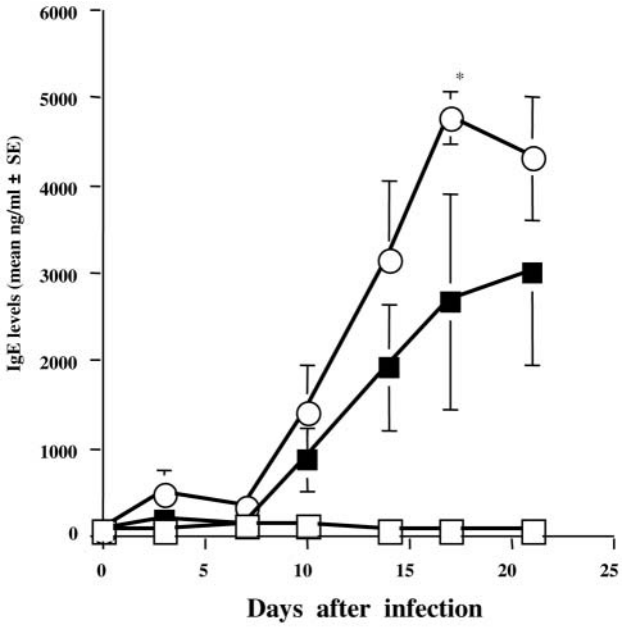

Figure 2. *Influence of Mesocestoides cortii infection on IgE hyper-production in mice. BALB/c mice were injected intraperitoneally with various numbers of M. cortii larvae on day 0. IgE levels in peripheral blood were examined 4, 7, 14, and 21 days after infection. Open square: non-infected; filled square: infected with 100 larvae; open circle: infected with 500 larvae. *Significant ($p<0.05$) as compared to 100 infection.*

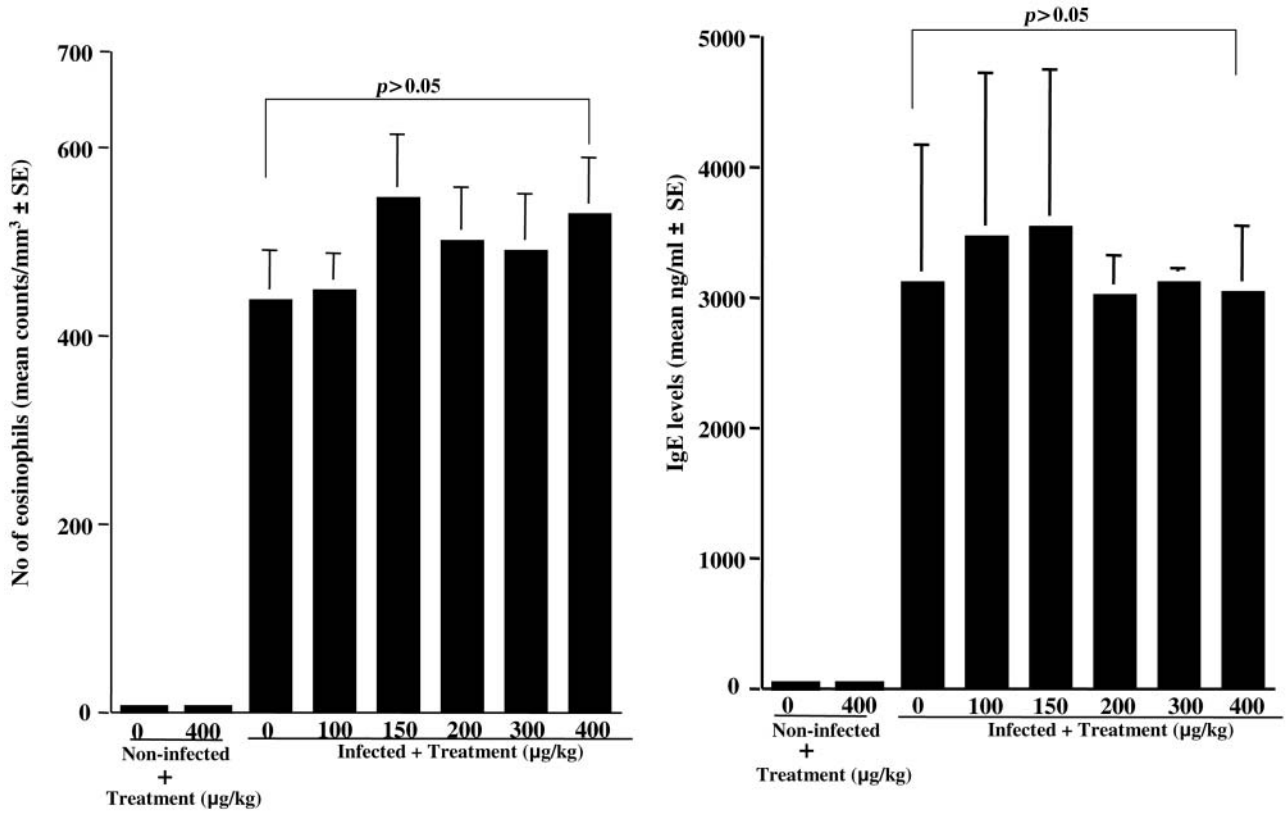

Figure 3. *Influence of epinastine hydrochloride on peripheral blood eosinophilia and IgE hyperproduction induced by Mesocestoides cortii infection in mice. BALB/c mice were injected intraperitoneally with 500 M. cortii larvae. The mice were treated orally with epinastine hydrochloride at a daily dose of 100 to 400 μg/kg, which was started on the day of infection. After 3 weeks, (a) the number of peripheral blood eosinophils and (b) IgE levels were examined.*

Discussion

EP is a well known second-generation antihistamine and is frequently used in the treatment and management of a wide variety of allergic disorders, including allergic rhinitis, urticaria and atopic dermatitis. Although its effect was mainly described from the perspective of a histamine receptor antagonist that affects mast cells (11), the precise therapeutic mechanisms on allergic diseases are not fully understood.

The most striking feature of the histopathology of allergic diseases is the intense infiltration of eosinophils, macrophages and lymphocytes (1). Of these, the eosinophil appears to be a key cell that has been associated with histological changes at the site of allergic immune responses and disease severity (1-5), suggesting that eosinophils may be important targets for the treatment and the management of allergic diseases. The experiments presented here characterized firstly the effects of EP on allergic immune responses, especially eosinophilia and IgE hyperproduction, during normal *in vivo* immune responses. The model used is that of eosinophilia and IgE hyperproduction during *M. cortii* infection. We examined the influence of EP on eosinophilia and IgE hyperproduction. The data obtained clearly show that EP cannot suppress these two immune responses induced by *M. cortii* infection, even when 300 to 400 μg/kg EP, almost equal to human therapeutic dose, was administered into experimental mice for three weeks.

Eosinophils can secrete a number of lipid mediators and basic proteins, which have been implicated in airway reactivity, vascular leak syndromes, destruction and sloughing of epithelium, and other inflammatory changes that underlie allergic diseases, including asthma and pollinosis (12, 13). Eosinophils also have the capacity to produce certain cytokines and chemokines such as granulocyte-colony stimulating factor, IL-5, RANTES and eotaxin, which exert an autocrine effect on eosinophil survival, differentiation and accumulation (12, 14). SCF is a primary cytokine involved in hematopoiesis, mast cell differentiation, and mast cell activation. It has also been shown to play a significant role in eosinophil-associated inflammatory responses (15). Therefore, the second part of the experiment examined the influence of EP on eosinophil

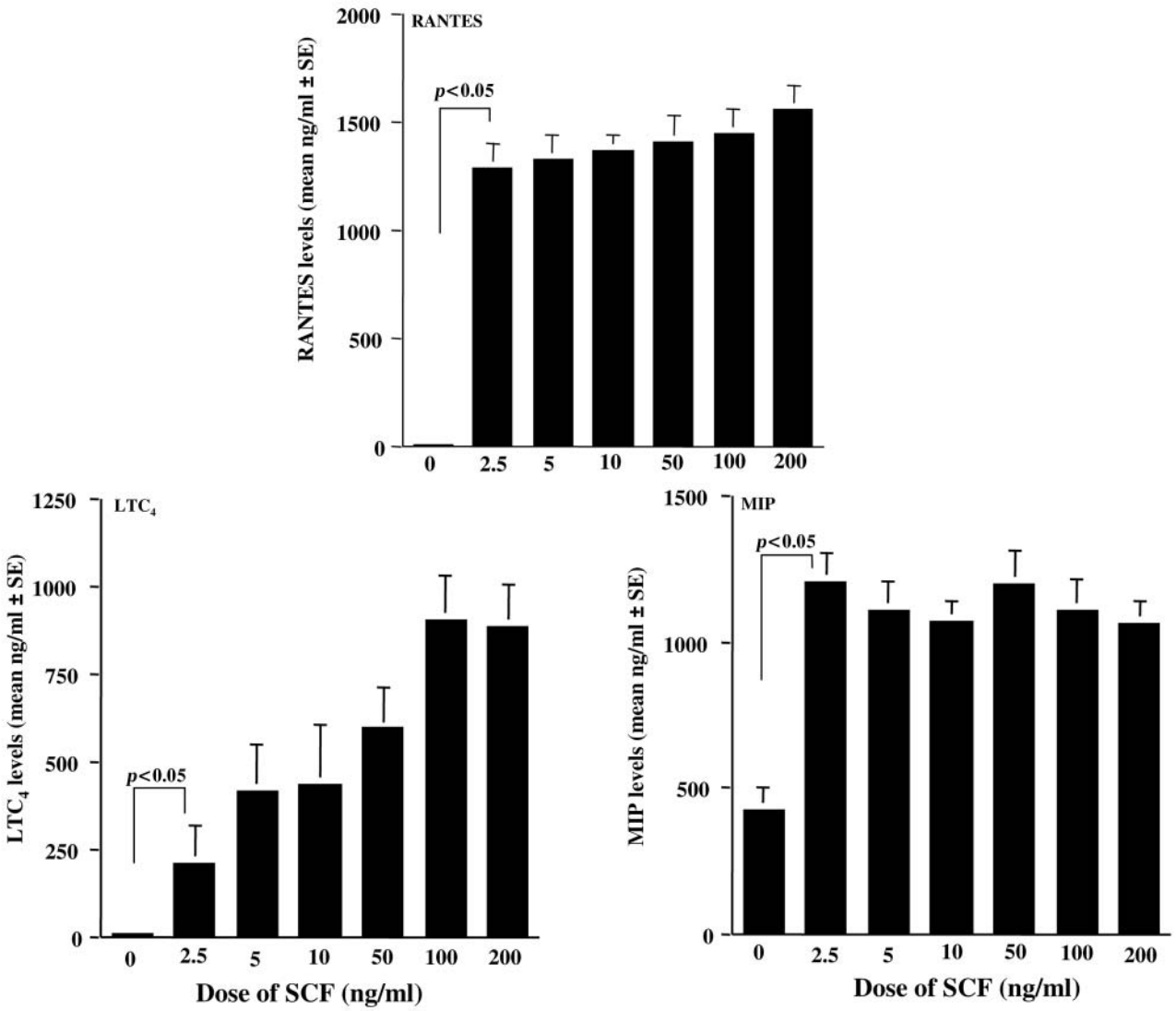

Figure 4. *Dose response profile of stem cell factor on eosinophil activation in vitro. Eosinophils (5x10^5 cells/ml) obtained from mice infected with Mesocestoides cortii (500 larvae/mouse) were stimulated with stem cell factor for 24 h. Factor levels in culture supernatants were examined by ELISA.*

activation *in vitro*. As assessed by examining the levels of RANTES, LTC_4 and MIP-1β, EP could suppress the production of these factors by eosinophils stimulated by SCF. A significant suppression of production was first observed at 25.0 ng/ml, which is equivalent to a therapeutic blood level (16). LTC_4 is a member of closely structurally related lipid molecules, Cysteinyl leukotrienes (CysLTs) was originally described as a slow-reacting substance of anaphylaxis (17). LTC_4 is synthetized from arachidonic acid by several types of cells (*e.g.* mast cells, eosinophils and macrophages, *etc.*) in response to many triggers, including receptor activation, antigen-antibody interaction, and physical stimuli such as cold (18). Any stimulation that increases intracellular calcium levels affects the production of LTC_4 (18). CysLTs have been shown to play a role in leucopoiesis, cellular migration from bone marrow to the circulation and adhesion of inflammatory cells to the vascular endothelium (17). It has also been reported that CysLTs enhance eosinophil migration and activation, and increase eosinophil survival time *in vitro* and *in vivo* (19). Taken together, the present results may suggest that EP exerts its attenuating effect on the clinical status of allergic diseases through the suppression of LTC_4 production by eosinophils.

Allergic inflammatory responses are characterized by structural abnormalities, including thickening of basement membrane and structural fibrosis associated with intense infiltration of eosinophils and macrophages (20, 21). Tissue

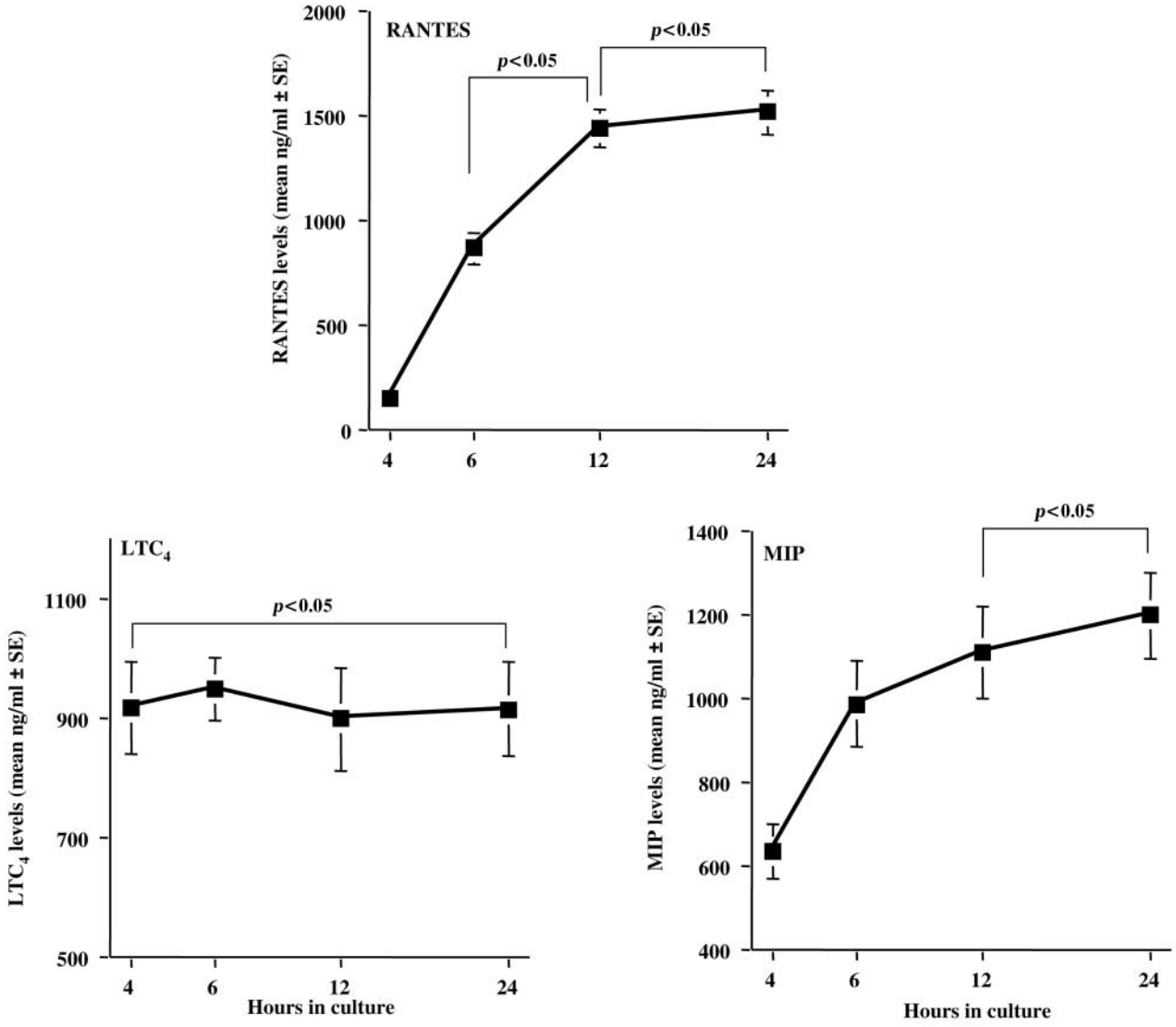

Figure 5. *Time course of eosinophil activation induced by stem cell factor stimulation in vitro. Eosinophils (5x10^5 cells/ml) obtained from mice infected with Mesocestoides cortii (500 larvae/mouse) were stimulated with stem cell factor at a concentration of 200 ng/ml. The culture supernatants were obtained after stimulation, and factor levels were assayed by ELISA.*

eosinophilia is due to a combination of specific and coordinated cellular processes appearing at different stages of eosinophil extravasation including adhesion, chemotaxis, and activation (1). Although IL-5 is responsible for eosinophil migration, *in vitro* and *in vivo* studies demonstrated specificity of chemokines, RANTES and eotaxin, for attraction and activation of eosinophils, and imply their participation in the specific recruitment of eosinophils to the site of allergic inflammation (22, 23). MIP-1β is a member of the CC subfamily of chemokines, which induce the migration and recruitment of monocytes and T cells to the sites of inflammation (24). MIP-1β has also been reported to enhance macrophage effector functions by inducing the production of nitric oxide, which is the most important final effector molecule in inflammatory diseases (25). Judging from these reports, the present results showing the suppressive activity of EP at therapeutic blood levels on RANTES and MIP-1β production provide possible mechanisms that could explain the favorable effects of EP on allergic diseases.

Although the present results clearly indicate one therapeutic mode of action of EP on eosinophil-mediated allergic immune responses, the precise mechanisms by which EP could suppress eosinophil activation by SCF stimulation

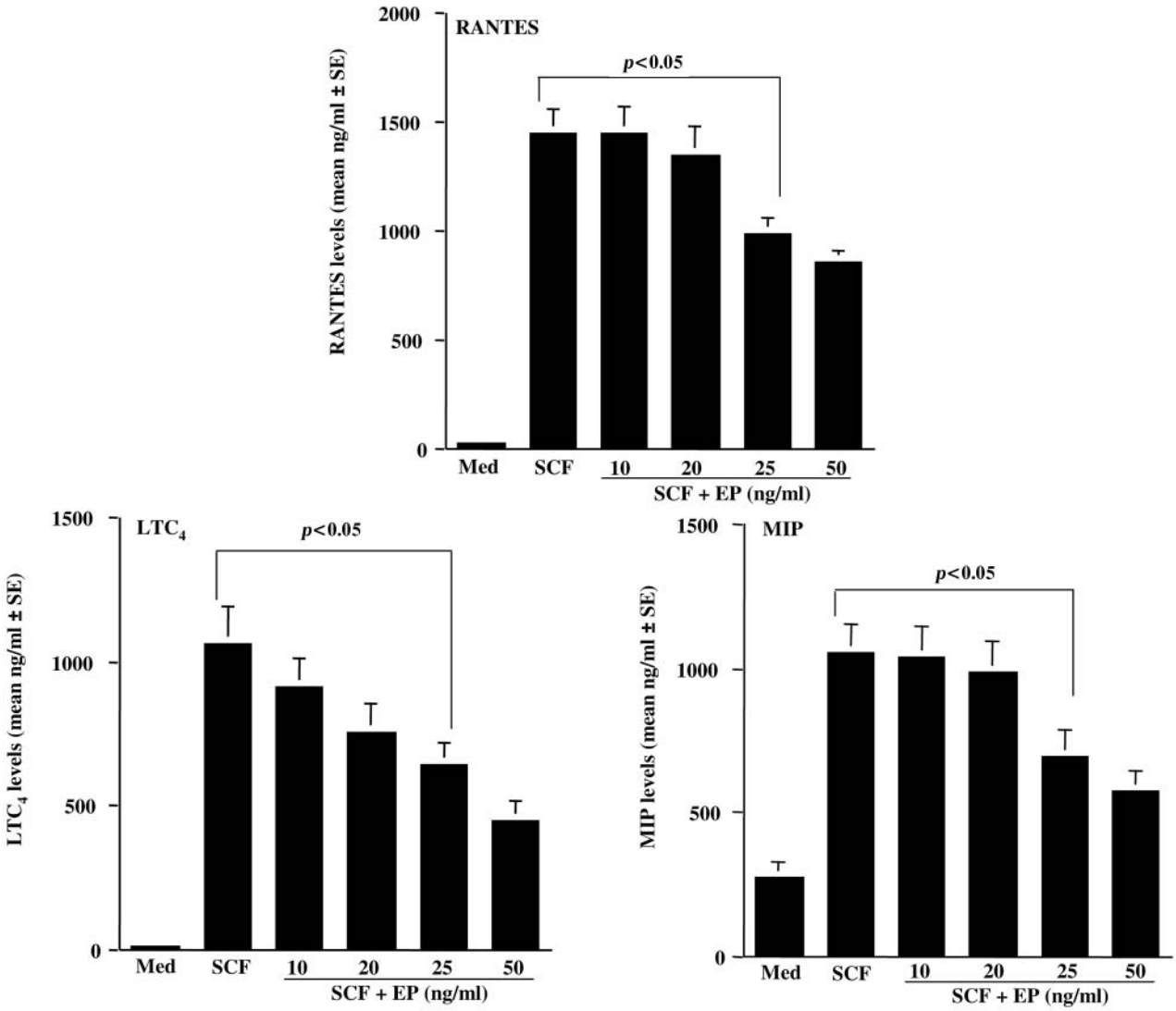

Figure 6. *Influence of epinastine hydrochloride (EP) on eosinophil activation induced by stem cell factor stimulation in vitro. Eosinophils ($5x10^5$ cells/ml) obtained from mice infected with Mesocestoide cortii (500 larvae/mouse) were stimulated with 200 ng/ml of stem cell factor (SCF) in the presence of different concentrations of EP for 24 h. Factor levels in culture supernatants were examined by ELISA.*

are not fully understood. SCF exerts its biological effect through a specific interaction with the cell surface receptor c-kit, which is a member of the receptor tyrosine kinase family (15). The SCF and c-kit complex has been reported to enhance the ability of inflammatory cells, such as mast cells and eosinophils, to produce a wide variety of chemokines (*e.g.* TARC and RANTES, *etc.*) through Ca^{2+}-dependent mechanisms (26). It has also been reported that the synthesis of CysLTs including LTC_4 requires an increase in intracellular calcium content (18). Several studies have demonstrated that H_1-receptor antagonists can prevent not only Ca^{2+} influx but also Ca^{2+} release from the intracellular Ca^{2+} store, which is responsible for the inhibition of both mediator release and signal transduction pathways that are related to mediator production (27-29). From the present results, it may be inferred that EP maintains a stable Ca^{2+} concentration in the cytosol and results in the inhibition of production (or release) of the mediators examined.

In conclusion, the present results suggest that some of the therapeutic effects of EP on allergic diseases depend on its ability to inhibit eosinophil activation.

Acknowledgements

This study was supported, in part, by Nihon-Boehringer Ingelheim Co. Ltd. (Tokyo, Japan)

References

1 Hogan SP: Recent advances in eosinophil biology. Int Arch Allergy Immunol *143(Suppl 1)*: 3-14, 2007.

2 Gleich GJ: Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol *105*: 651-663, 2000

3 Widegren H, Korsgren M, Andersson M and Greiff L: Effects of TNFalpha on the human nasal mucosa *in vivo*. Respir Med *101*: 1982-1087, 2007.

4 Erjefalt JS, Greiff L, Andersson M, Matsson E, Petersen H, Linden M, Ansari T, Jeffery PK and Persson CGA: Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. Am J Respir Crit Care Med *160*: 304-312, 1999.

5 Davoine F, Ferland C, Chakir J, Lee JE, Adamko DJ, Moqbel R and Laviolette M: Interleukin-12 inhibits eosinophil degranulation and migration but does not promote eosinophil apoptosis. Int Arch Allergy Immunol *140*: 277-284, 2006.

6 Hur GY, Lee SY, Lee SH, Kim SJ, Lee KJ, Jung JY, Lee EJ, Kang EH, Jung KH, Lee SY, Kim JH, Shin C, Shim JJ, In KH, Kang KH and Yoo SH: Potential use of an anticancer drug gefinitib, an EGFR inhibitor, on allergic inflammation. Exp Mol Med *39*: 367-375, 2007.

7 Asano K, Sugimoto K, Watanabe T, Hisano M and Hisamitsu T: Suppressive effects of the Chinese herbal remedy *Tripterygium wilfordii* Hook f on eosinophilia and IgE hyperproduction in mice. Allergol Int *49*: 37-45, 2000.

8 Dold S, Heinrich J, Wichmann EH and Wist M: Ascaris-specific IgE and allergic sensitization in a cohort of school children in the former East Germany. J Allergy Clin Immunol *102*: 414-420, 1998.

9 The Physiological Society of Japan: Guidline Principles for the Care and Use of Animals in the Field of Physiological Science. J Physiol Society Japan *51*: 289-290, 1990.

10 Korenaga M, Hitoyoshi Y, Takatsu K and Tada I: Cross-resistance between *Strongyloides benezuelensis* and *S. ratti* in mice. J Helminthol *69*: 119-123, 1995.

11 Tasaka K: Epinastine: an update of its pharmacology, metabolism, clinical efficacy and tolerability in the treatment of allergic diseases. Drug Today *36*: 735-757, 2000.

12 Tamachi T, Watanabe N, Oya Y, Kagami S, Hirose K, Saito Y, Iwamoto I and Nakajima H: B and T lymphocyte attenuator inhibits antigen-induced eosinophil recruitment into the airways. Int Arch Allergy Immunol *143(Suppl 1)*: 50-55, 2007.

13 Schleimer RP and Bochner BS: The effects of glucocorticoids on human eosinophils. J Allergy Clin Immunol *94*: 1202-1203, 1994.

14 Conroy DM and Williams TJ: Eotaxin and the attraction of eosinophils to the asthmatic lung. Respir Res *2*: 150-156, 2001.

15 Oliveira SHP, Taub DD, Nagel J, Smith R, Hogaboam CM, Berlin A and Lukacs NW: Stem cell factor induces eosinophil activation and degranulation: mediator release and gene array analysis. Blood *100*: 4291-4297, 2002.

16 Sarashina A, Tatami S, Yamamura N, Tsuda Y and Igarashi T: Population pharmacokinetics of epinastine, a histamine H_1 receptor antagonist, in adults and children. Br J Clin Pharmacol *59*: 43-53, 2005.

17 Ogawa Y and Calhoun WJ: The role of leukotrienes in airway inflammation. J allergy Clin Immunol *118*: 789-798, 2006.

18 Salvi SS, Krishna MT, Sampson AP and Holgate ST: The anti-inflammatory effects of leukotrienes-modifying drugs and their use in asthma. Chest *119*: 1533-1546, 2001.

19 Dranz JM, Israel E and O'Byrne PM: Treatment of asthma with drugs modifying the leukotriene pathway. N Engl J Med *340*: 197-206, 1999.

20 Calderon MA, Lozewicz S, Prior A, Jordan S, Trigg CJ and Davies RJ: Lymphocyte infiltration and thickness of the nasal mucus membrane in perennial and seasonal allergic rhinitis. J Allergy Clin Immunol *93*: 635-643, 1994.

21 Oyamada H, Kamada Y, Saito N, Tsuda A, Urayama O, Yamada H, Hirasawa H, Yamaguchi K, Ueki S and Chihara J: RANTES production from mononuclear cells in response to the specific allergen in asthma patients. Allergol Int *55*: 253-259, 2006.

22 Niimi K, Asano K, Shiraishi Y, Nakajima T, Wakaki M, Kagyo J, Takihara T, Suzuki Y, Fukunaga K, Shiomi T, Oguma T, Sayama K, Yamaguchi K, Natori Y, Matsumoto M, Seya T, Yamaya M and Ishizaka A: TLR-3 mediated synthesisi and release of eotaxin-1/CCL11 from human bronchial smooth muscle cells stimulated with double-strand RNA. J Immunol *178*: 489-495, 2007.

23 Tahan F, Karaaslan C, Aslan A, Kiper N and Kalayci O: The role of chemokines in exercised-induced bronchoconstriction in asthma. Ann Allergy Asthma Immunol *96*: 819-825, 2006.

24 Haelens A, Wuyts A, Proost P, Struyf S, Opedenaller G and van Damme J: Leukocyte migration and activation by murine chemokines. Immunobiol *195*: 499-521, 1996.

25 Aliberti JC, Machado FS, Souto JT, Campanelli AP, Teixeira MM, Gazzinelli RT and Silva JS: Beta-chemokines enhance parasite uptake and promote nitric oxide-dependent microbiostatic activity in murine inflammatory macrophages infected with *Trypanosoma cruzi.* Infect Immun *67*: 4819-4826, 1999.

26 Berridge MJ and Irvine RF: Inositol phosphate and cell signaling. Nature *341*: 197-205, 1989.

27 Gupta S, Banfield C, Kanteesaria B, Marino M, Clement R, Affrime M and Batra V: Pharmacokinetics and safety profile of desloratadine and fexofenadine when coadministered with azithromycine: A randomized, placebo-controlled, parallel-group study. Clin Ther *23*: 451-466, 2001.

28 Gelfand EW, Cui ZH, Takeda K, Kanehiro A and Joetham A: Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponssiveness. J Allergy Clin Immunol *110(Suppl 4)*: S85-95, 2002.

29 Masuo M, Shimada T and Kitazawa T: Mechanism of inhibitory effect of azelastine on muscle contraction. J Pharmacol Exp Ther *26*: 1300-1308, 1992.

Received July 2, 2007
Revised November 2, 2007
Accepted December 3, 2007