



Traditional Chinese medicine for atopic eczema: PentaHerbs formula suppresses inflammatory mediators release from mast cells

Ben Chung Lap Chan^a, Kam Lun Ellis Hon^b, Ping Chung Leung^a, Sze Wing Sam^c, Kwok Pui Fung^a, Mavis Yuk Ha Lee^a, Hang Yung Alaster Lau^{c,*}

^a Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

^b Department of Paediatrics, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

^c Department of Pharmacology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

ARTICLE INFO

Article history:

Received 31 March 2008

Received in revised form 25 July 2008

Accepted 26 July 2008

Available online 3 August 2008

Keywords:

Atopic eczema

Mast cells

Traditional Chinese medicine

Histamine release

ABSTRACT

Background: PentaHerbs formula (PHF) containing *Cortex Moutan*, root bark of *Paeonia suffruticosa* Andr. (Ranunculaceae), *Cortex Phellodendri*, bark of *Phellodendron chinensis* Schneid. (Rutaceae), *Flos Lonicerae*, flower of *Lonicera japonica* Thunb. (Capri-foliaceae), *Herba Menthae*, aerial part of *Mentha haplocalyx* Briq. (Labiatae) and *Rhizoma Atractylodis*, rhizome of *Atractylodes lancea* (Thunb.) DC. (Compositae) at the ratio of 2:2:2:1:2 was useful in the management of eczema.

Aim of the study: Since the mechanism of action of PHF is not known, we aimed to investigate the actions of PHF on mast cell activation.

Materials and methods: Effects of aqueous extracts of PHF and individual component herb on mediator release from rat peritoneal mast cells (RPMCs) and cytokine production from HMC-1 were investigated. **Results:** PHF, *Cortex Moutan* and *Herba Menthae* significantly attenuated histamine release and prostaglandin D₂ synthesis from RPMC activated by anti-IgE and compound 48/80 ($p < 0.05$). While *Flos Lonicerae* and *Rhizoma Atractylodis* suppressed only mediator release from compound 48/80 activated RPMC, *Cortex Phellodendri* potentiated only anti-IgE induced mediator release ($p < 0.05$). However, with the exception of *Cortex Moutan*, PHF and the other four component herbs failed to affect cytokine production in HMC-1.

Conclusions: Although individual herbs demonstrated different modulating effects on mast cells, inhibition of inflammatory mediator release from mast cells would contribute to the therapeutic efficacy of PHF.

© 2008 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Atopic eczema (AE) is a common chronic relapsing disease with high prevalence in children (Leung et al., 2007). It has been estimated that 15% of school children aged 13–14 years have a history of AE in Hong Kong (Leung et al., 1997). Inasmuch as the etiology of AE is only partially understood, treatment is empirical and is based on the use of topical or systemic corticosteroids aimed at reducing skin inflammation (Leung, 2000). As there is no definitive cure for the condition among Western medicines, there has been considerable interest in the use of traditional Chinese medicine (TCM) as a potential adjunct therapy for AE. This prompted us to investigate the effect of TCM intervention for the treatment of AE. PentaHerbs formula (PHF) is an ancient Chinese concoction which contains *Cortex Moutan*, the dried root bark of *Paeonia suffruticosa*

Andr. (Ranunculaceae), *Cortex Phellodendri*, the dried bark of *Phellodendron chinensis* Schneid. (Rutaceae), *Flos Lonicerae*, the dried flower of *Lonicera japonica* Thunb. (Capri-foliaceae), *Herba Menthae*, the dried aerial part of *Mentha haplocalyx* Briq. (Labiatae) and *Rhizoma Atractylodis*, the dried rhizome of *Atractylodes lancea* (Thunb.) DC. (Compositae) at the proportion of 2:2:2:1:2. In our previous randomized, double-blind, placebo-controlled studies in 85 children with AE (Hon et al., 2004, 2007b), it was found that PHF is efficacious in improving quality of life and reducing topical corticosteroid use in children with moderate-to-severe AE. The PHF was proposed to work according to the following TCM principles: (i) *Flos Lonicerae* and *Herba Menthae* clear damp-heat from the exterior, (ii) *Cortex Moutan* clears heat from blood, and (iii) *Rhizoma Atractylodis* and *Cortex Phellodendri* clear damp-heat from the interior. These herbs possess anti-allergic, anti-inflammatory, anti-pruritic and sedative properties and have been extensively used in China for the treatment of allergic diseases including AE, asthma and allergic rhinitis (Hon et al., 2006). We have so far demonstrated that the PHF could dose dependently inhibit peripheral blood mononuclear

* Corresponding author. Tel.: +852 2609 6846; fax: +852 2603 5139.

E-mail address: hyalau@cuhk.edu.hk (H.Y.A. Lau).

cells proliferation and brain-derived neurotrophic factor secretion upon staphylococcal enterotoxin B stimulation (Leung et al., 2008) and these in vitro immunomodulatory properties might account for the clinical benefits in AE patients.

Mast cells are well known for their critical roles in allergic diseases which are attributable to their potent capability to produce multiple proinflammatory mediators including preformed granular contents such as histamine, newly synthesized lipid mediators such as prostaglandin D₂ (PGD₂) and cytokines (GM-CSF, IL-6 and TNF α) after activation (Wang and Lau, 2006). Subsequent to activation by antigens, cytokines, growth factors or hormones, chemical mediators released from skin mast cells cause immediate skin inflammatory reactions including local vasodilation, T-cell recruitment and itching (Theoharides et al., 2007). In addition to the progression of the immediate inflammatory response, the release of these mediators also initiates the onset of the late-phase inflammatory responses. Inhibition of mast cell activation in skin is thus a potential therapeutic strategy in AE treatment.

Although our recent randomized study showed PHF could reduce the use of topical corticosteroid and improve the quality of life in patients with AE, this treatment did not result in significant decrease in their AE severity (Hon et al., 2007b). We hypothesize that the dosage of PHF in the clinical trial might be sub-therapeutic, or the ratio of the herbal materials had not been optimized. We hence proposed to further investigate the modulating effects of PHF on inflammatory mediator release and cytokine production from mast cells for the better understanding of the underlying mechanisms of the observed clinical efficacy on AE treatment. The rat peritoneal mast cell (RPMC) is a good model for investigating the contribution of inflammatory mediators released by mast cell degranulation in skin related diseases as it is functionally comparable to human skin mast cells in their responses to substance P and compound 48/80 (Mousli et al., 1994; Emadi-Khiav et al., 1995) while the human mast cell line (HMC-1) is appropriate for investigating the release of cytokines from mast cells.

2. Materials and methods

2.1. Sources and authentication of herbal materials

The PentaHerbs formula consists of five herbal components: *Cortex Moutan* (the dried root bark of *Paeonia suffruticosa* Andr. in the family of Ranunculaceae), *Cortex Phellodendri* (the dried bark of *Phellodendron chinensis* Schneid. in the family of Rutaceae), *Flos Lonicerae* (the dried flower of *Lonicera japonica* Thunb. in the family of Capri-foliaceae), *Herba Menthae* (the dried aerial part of *Mentha haplocalyx* Briq. in the family of Labiatae), and *Rhizoma Atractylodis* (the dried rhizome of *Atractylodes lancea* (Thunb.) DC. in the family of Compositae). All the raw herbs were purchased from the herbal wholesale market of Sheung Wan, Hong Kong. All five herbs were authenticated by a morphological expert, Dr. Cao Hui at the National Engineering Research Centre for Modernization of Traditional Chinese Medicine (Zhuhai, Guangdong, China). The authenticities of the herbs were further verified by thin layer chromatography according to the recommended method described in the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Committee, 2005) using reference compounds or reference herbs purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Herbarium voucher specimens were deposited at the Museum of the Institute of Chinese Medicine, the Chinese University of Hong Kong with voucher specimen numbers assigned: *Cortex Moutan* (2003–2456), *Cortex Phellodendri* (2006–3016), *Flos Lonicerae* (2007–3074), *Herba Menthae* (2005–2806), and *Rhizoma Atractylodis* (2005–2604).

2.2. Preparation of herbal extracts

PHF was prepared as previously described (Hon et al., 2006). Briefly, 20 g of *Cortex Moutan*, *Cortex Phellodendri*, *Flos Lonicerae*, *Rhizoma Atractylodis* and 10 g of *Herba Menthae* were refluxed in 900 ml of distilled water at 100 °C for 2 h and repeated twice. These three batches of water extracts were mixed together and centrifuged to remove the herbal debris. The combined extract was then vacuum dried and the resulting herbal powder was stored in desiccators until use. Same procedures were employed to prepare the extractions of individual herbs.

2.3. Animal sensitization and purification of rat peritoneal mast cells

All animals were handled in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and were approved by the Animal Research Ethnic Committee, The Chinese University of Hong Kong. Male Sprague–Dawley rats weighing 150–200 g were actively immunized by an intraperitoneal injection of 0.5 ml phosphate-buffered saline containing ovalbumin (0.5 mg), Al(OH)₃ (120 mg) and *Bordetella pertussis* (0.8 I.U.). Eight weeks later, the sensitized animals were sacrificed by decapitation after lightly anaesthetized with diethyl ether for the isolation of peritoneal mast cells. Full HEPES-buffered tyrode (FHB) at pH 7.4 (containing: NaCl 137 mM, KCl 2.7 mM, CaCl₂ 1 mM, NaH₂PO₄ 0.4 mM, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 10 mM, glucose 5.6 mM and MgCl₂ 1.2 mM) was used throughout the study. Mixed peritoneal cells were collected from each rat by peritoneal lavage with 20 ml of FHB supplemented with 1 mg/ml of bovine serum albumin (BSA-FHB). The cells were washed twice in ice-cold BSA-FHB by centrifugation (190 × g, 4 °C, 5 min), resuspended in 1 ml of ice-cold FHB, and then mixed with 4 ml of isotonic Percoll solution (SG = 1.017) in calcium-free FHB. Mast cells were purified by centrifugation (190 × g, 4 °C, 25 min) through the continuous density gradient generated by the Percoll and was pelleted at the bottom of the tube. Residual Percoll was eliminated by two washes in BSA-FHB and a final wash in FHB. The purified mast cells were finally resuspended in the required amount of FHB.

2.4. Mast cell incubation, histamine and PGD₂ assay

Aliquots of purified mast cells (250 μ l), which had been equilibrated in FHB at 37 °C for 10 min, were added to 200 μ l of FHB with herbal extracts. After incubating the cells with the herbal extracts for 5 min, 50 μ l of secretagogues including goat anti-rat IgE (ICN, 1:300 dilution) or compound 48/80 (Sigma, 0.1 μ g/ml) were added. The cells were further incubated at 37 °C for 15 min after the addition of secretagogues and the reaction was terminated by the addition of ice-cold FHB. The supernatant and cell pellet were then separated by centrifugation (190 × g, 4 °C, 5 min). Histamine contents in mast cell pellets and supernatants were measured spectrofluorometrically using a Bran + Luebbe Auto-Analyzer 2 (Hamburg, Germany). The percentages of total cellular content of histamine (sum of supernatant content and cell pellet content) released into the supernatant following different treatments were then calculated. Prostaglandin D₂ was measured using a commercial EIA kit of detection limits of 3.1 pg/ml (Cayman Chemical, USA).

2.5. Human mast cell line HMC-1 culture and cytokine release studies

The human mast cell line HMC-1 was a generous gift from Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN, USA). It was maintained

in Iscove's modified Dulbecco's media (IMDM), supplemented with 10% fetal bovine serum (FBS), 2 mmol/l L-glutamine, 100 U/ml penicillin G and 10 mg/ml streptomycin (Gibco, Grand Island, NY, USA), and 100 μ M α -thioglycerol (Sigma, St. Louis, USA). For cytokine release studies, HMC-1 (10^6) were pre-incubated for 30 min with 500 μ g/ml of PHF or dexamethasone (1 μ M), followed by a 24-h incubation with a mixture of calcium ionophore A23187 (1 μ M) and phorbol-12 myristate 13-acetate (50 nM) (A23187 + PMA, Sigma, St. Louis, USA). The cell free supernatants were then assayed for GM-CSF, IL-6, IL-8, IL-10 and TNF- α production by commercially available human cytokine ELISA kits (BD OptEIA, USA) according to the manufacturer instruction with detection limits ranged from 3.1 to 7.8 pg/ml.

2.6. Expression of results and statistical analyses

In each experiment, the spontaneous release of histamine or prostaglandin in buffer alone was subtracted from all measurements. Results on rat peritoneal mast cells are expressed as the percentages of control release induced by the secretagogue (anti-IgE or compound 48/80) alone, i.e., histamine release (% of control) or prostaglandin release (% of control), whereas results on HMC-1 are expressed as the actual levels of cytokines released. The levels of mediator/cytokine release induced by the secretagogue in the presence of herbal ingredients were compared with the control levels of mediator/cytokine release induced by the secretagogue alone. Statistical analyses were performed using the Student's *t*-test and differences were considered significant when the probability (*p*) was <0.05. All data are mean $\pm$ standard error of mean (S.E.M.) for *n* independent observations.

3. Results

3.1. Effects of PHF on anti-IgE and compound 48/80 activated RPMC

Rat peritoneal mast cells spontaneously released undetectable level of PGD₂ and less than 15% of total cellular histamine when incubated in buffer alone and none of the tested herbal extracts affected these levels of spontaneous mediator release. Anti-IgE and compound 48/80 alone induced $44.2 \pm 4.1\%$ and $58.9 \pm 2.3\%$ of histamine release and 2598 ± 280 pg/10⁶ cells and 2642 ± 536 pg/10⁶ cells of PGD₂ production, respectively. As shown in Figs. 1 and 2, PHF significantly inhibited both histamine release and PGD₂ production from RPMC stimulated by anti-IgE and compound 48/80 dose-dependently from 500 to 1000 μ g/ml (*p* < 0.05). PHF was more efficacious in inhibiting histamine release and PGD₂ production in mast cells that were activated by compound 48/80 than those activated by anti-IgE. At the highest concentration of PHF tested (1000 μ g/ml), anti-IgE induced histamine release and PGD₂ production were reduced by $46.9 \pm 5.9\%$ and $54.9 \pm 11.7\%$, respectively, while compound 48/80 induced histamine release and PGD₂ production were reduced by $71.4 \pm 3.5\%$ and $84.9 \pm 2.9\%$, respectively.

3.2. Effects of the components of PHF on anti-IgE activated RPMC

The five components of PHF demonstrated different actions on anti-IgE induced activation of RPMC (Fig. 1). *Herba Menthae* did not demonstrate any significant modulating actions. However, *Cortex Moutan*, *Flos Lonicerae* and *Rhizoma Atractylodis* demonstrated significant suppression while *Cortex Phellodendri* showed significant potentiating action on anti-IgE-induced release of histamine and PGD₂ (*p* < 0.05). *Cortex Moutan* reduced the levels of histamine and PGD₂ release by $90.8 \pm 0.6\%$ and $82.4 \pm 3.5\%$, respectively, at 1000 μ g/ml while *Flos Lonicerae* and *Rhizoma Atractylodis*

suppressed less than 50% of anti-IgE-induced mediator release at 1000 μ g/ml. In contrast, *Cortex Phellodendri*, significantly enhanced the anti-IgE-induced histamine release and PGD₂ production from RPMC by up to $41.1 \pm 13.1\%$ and $26.9 \pm 16.1\%$, respectively, at 1000 μ g/ml.

3.3. Effects of the components of PHF on compound 48/80 activated RPMC

With the exception of *Cortex Phellodendri* which did not produce significant modulating action, all other components could dose dependently inhibit histamine release from compound 48/80 activated RPMC (Fig. 2a, *p* < 0.05). The release of PGD₂ was also significantly suppressed by the same herbs but in a less dose dependent manner (Fig. 2b, *p* < 0.05). *Cortex Moutan* and *Herba Menthae* were significantly more efficacious suppressors of compound 48/80 induced histamine release than the other two components *Flos Lonicerae* and *Rhizoma Atractylodis*. At the maximum concentration of 1000 μ g/ml tested, more than 90% of compound 48/80 induced histamine release was reduced by *Cortex Moutan* and *Herba Menthae* while not more than 70% of compound 48/80 induced histamine release was reduced by *Flos Lonicerae* and *Rhizoma Atractylodis*. When tested against compound 48/80 induced PGD₂ production, *Cortex Moutan* continued to be the most potent suppressor while *Herba Menthae* shared similar potency as *Flos Lonicerae* and *Rhizoma Atractylodis*.

3.4. Effects of PHF components on cytokines production of HMC-1

In contrast to the positive control, dexamethasone (1 μ M), PHF (500 μ g/ml) has no significant effect on the productions of cytokines from HMC-1 activated by A23187 + PMA (Fig. 3). When the effects of individual component herbs of PHF were investigated at 500 μ g/ml, only *Cortex Moutan* could significantly inhibit the productions of GM-CSF, IL-6, IL-8, IL-10 and TNF α productions (Fig. 3, *p* < 0.05). The levels of suppression produced by *Cortex Moutan* were not significantly different from those produced by dexamethasone.

4. Discussion

Mast cells are located in tissues that interface with the external environment including the skin and the number of mast cells is increased during the early phase of AE (Theoharides et al., 2007). The mediators released from activated mast cells, including histamine, prostaglandins and cytokines contribute to pruritus and inflammation in AE (Alenius et al., 2002). Besides immunological trigger, which involves the aggregation of cell surface high-affinity receptors for IgE (Fc ϵ RI) by their corresponding antigens, activation of mast cells can be achieved by basic secretagogues such as substance P or polycationic compounds (e.g., compound 48/80 and naturally occurring polyamines) via the peptidergic pathway (Shefler et al., 2008). Serum levels of substance P are associated with the disease severity of AE (Hon et al., 2007a), and an elevated level of substance P may contribute to the progression of skin inflammation through activation of mast cells. While PHF can significantly suppress the release of preformed (histamine) and newly synthesized (PGD₂) mediators from both anti-IgE and compound 48/80 activated mast cells, the formulation is more potent against the latter secretagogue. This mast cell stabilizing effect of PHF may contribute to the clinical effectiveness in AE through suppression of the initial allergen induced immediate acute inflammation and the subsequent chronic inflammation sustained by elevated level of substance P released from nerve endings.

The preference of PHF in inhibiting compound 48/80 induced mast cell activation in general is reflected in the mast cell modu-

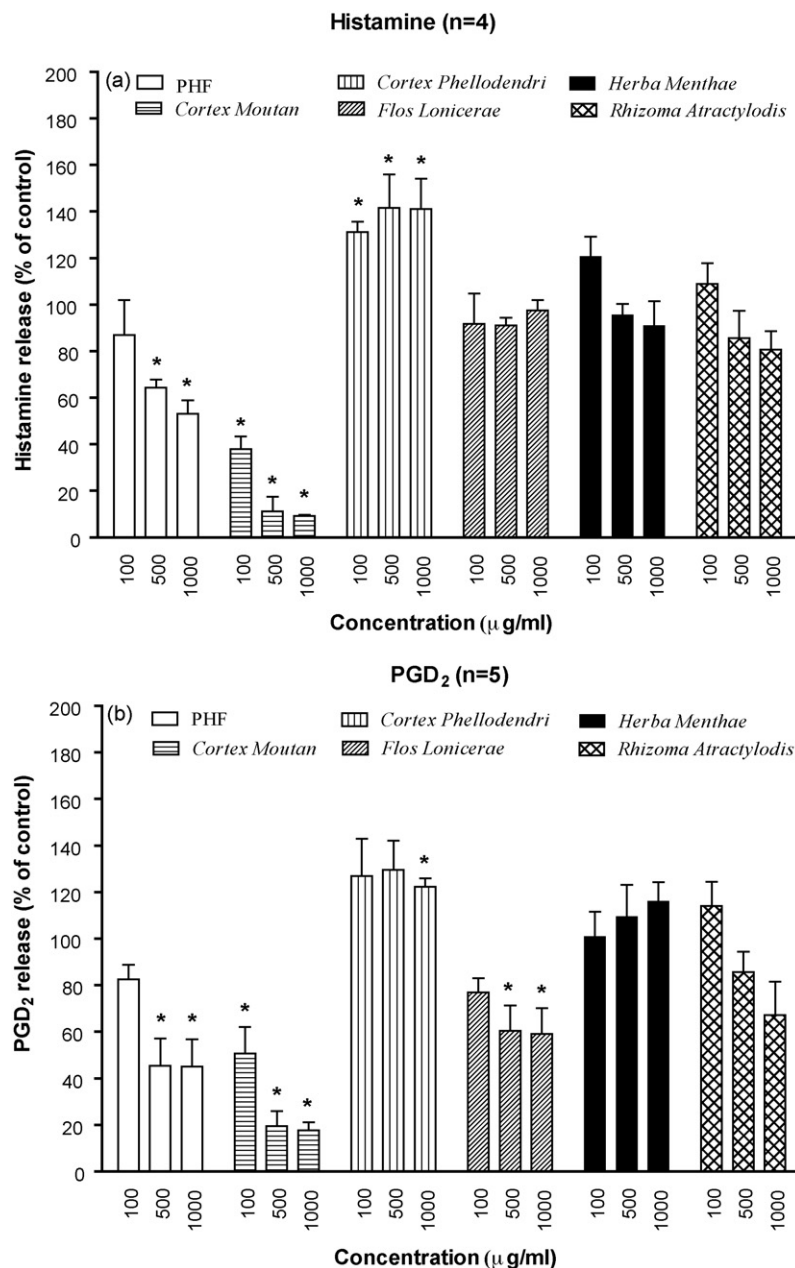


Fig. 1. Effects of PHF and individual components: *Cortex Moutan*, *Cortex Phellodendri*, *Flos Lonicerae*, *Herba Menthae* and *Rhizoma Atractylodis* on (a) histamine release and (b) PGD₂ production from anti-IgE activated rat peritoneal mast cells. Spontaneous histamine release was $10.9 \pm 0.7\%$ while anti-IgE induced histamine and PGD₂ release were $44.2 \pm 4.1\%$ and 2598 ± 280 pg/ 10^6 cells, respectively. Results are expressed as percentages of control mediator release induced by anti-IgE (1:300 dilution) and are given as mean $\pm$ S.E.M. for $n = 4$ or 5 . *Indicates $p < 0.05$ when compared with the control level of mediator release induced by anti-IgE alone.

lating actions of the individual component herbs. *Herba Menthae* demonstrated distinct differential suppression of mast cell mediator release stimulated by compound 48/80 while ineffective against those induced by anti-IgE. Although *Cortex Moutan*, *Flos Lonicerae* and *Rhizoma Atractylodis* demonstrated suppression of mediator release from mast cells activated by both secretagogues, the levels of suppression of compound 48/80 induced mast cell activation were significantly higher. In contrast to IgE receptor mediated mast cell activation which involves initially the activation of tyrosine kinases and an obligatory extracellular Ca^{2+} influx, the basic secretagogues are believed to directly stimulate a pertussis toxin-sensitive intracellular heterotrimeric G proteins of the G_i or G_o subtypes without the involvement of a membrane receptor (Mousli et al., 1994; Emadi-Khiav et al., 1995). This G protein is believed

to locate downstream of phospholipase C (PLC) activation and the site of actions of Ca^{2+} (Lillie and Gomperts, 1992) and thus activation of mast cells by compound 48/80 can occur in the absence of PLC activation and the subsequent elevations in cytosolic Ca^{2+} (Aridor et al., 1993). It is hence likely that *Herba Menthae* specifically interferes with the activation of this G protein or the subsequent biochemical pathways while the other PHF components, with the exception of *Cortex Phellodendri*, interfere more potently with this non-immunological pathway than the IgE receptor mediated pathway.

In contrast to the general mast cell inhibitory actions of PHF and the other four component herbs, *Cortex Phellodendri* demonstrated an opposing effect which may hamper the effectiveness of PHF in the clinical treatment of allergic disease. The enhancement

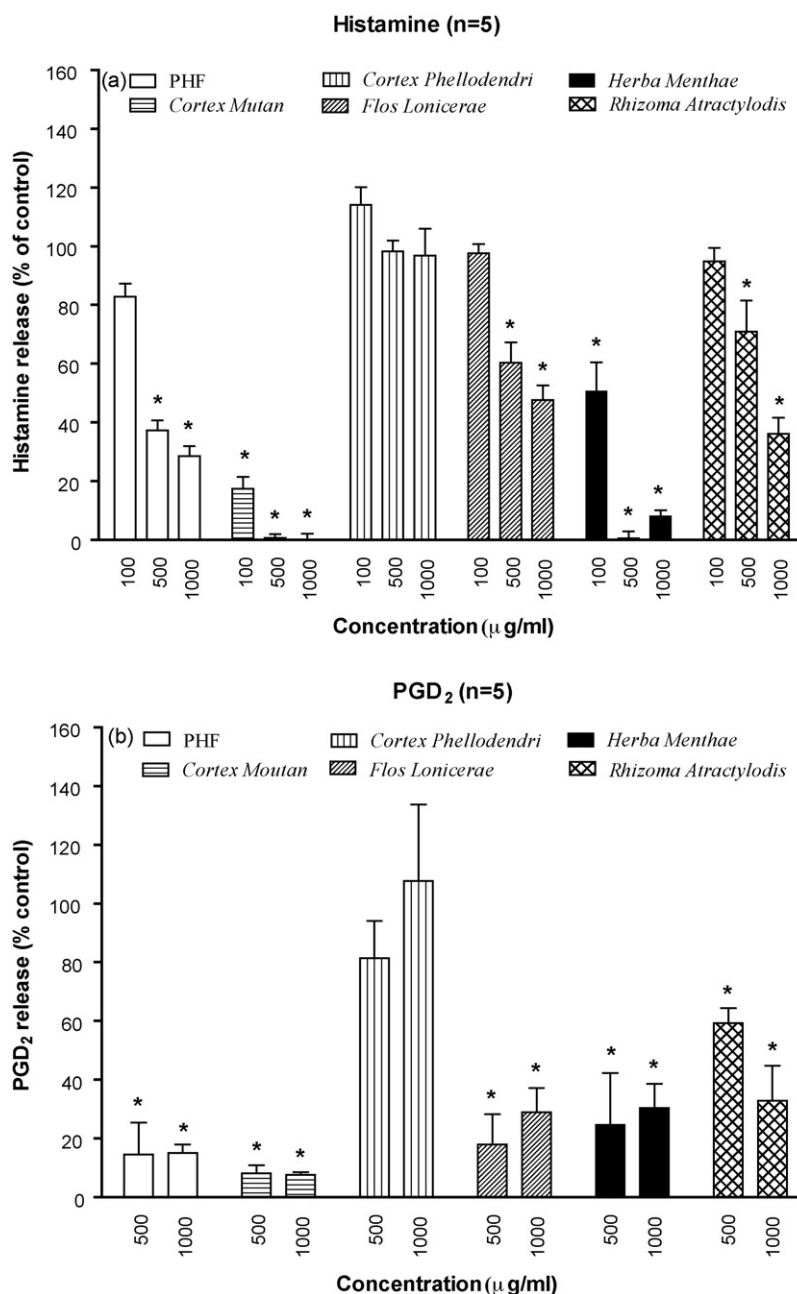


Fig. 2. Effects of PHF and individual components: *Cortex Moutan*, *Cortex Phellodendri*, *Flos Lonicerae*, *Herba Menthae* and *Rhizoma Atractylodis* on (a) histamine release and (b) PGD₂ production from compound 48/80 activated rat peritoneal mast cells. Spontaneous histamine release was $12.2 \pm 1.0\%$ while compound 48/80 induced histamine and PGD₂ release were $58.9 \pm 2.3\%$ and $2642 \pm 536 \text{ pg}/10^6$ cells, respectively. Results are expressed as percentages of control mediator release induced by compound 48/80 (0.1 μg/ml) alone and are given as mean $\pm$ S.E.M. for $n = 5$. *Indicates $p < 0.05$ when compared with the control level of mediator release induced by compound 48/80 alone.

of mast cell mediators release by this herb, however, is specific only for IgE receptor mediated activation since similar activity was not observed with compound 48/80 induced mediator release. Although *Cortex Phellodendri* does not seem to contribute immediately to the clinical efficacy of PHF, the inclusion of this herb in the original formula may serve the purpose of retaining some level of positive immunomodulatory actions for maintaining the normal defense functions of the immune system since the herb preferentially enhances IgE mediated mast cell activation. This somehow illustrates the polypharmacy approach of traditional Chinese medicines which aims at maintaining homeostasis during treatments.

In addition to the inflammatory mediators, mast cells are also an important source of cytokines, such as IL-6, IL-10, GM-CSF and TNF- α , which have been implicated in the pathogenesis of AE. Since clinical use of PHF has been demonstrated to reduce the frequency of steroid use, it was quite unexpected to find that 500 μg/ml of PHF, which significantly suppressed the release of inflammatory mediators from RPMC, failed to demonstrate any modulating effects on cytokine release from the human mast cell line HMC-1 activated by A23187 + PMA. The discrepancy may be due to the fact that HMC-1 is an atypical mast cell like cell line which does not possess intact functional IgE receptors and peptidergic activation mechanism and thus cannot truly represent the responses of skin mast

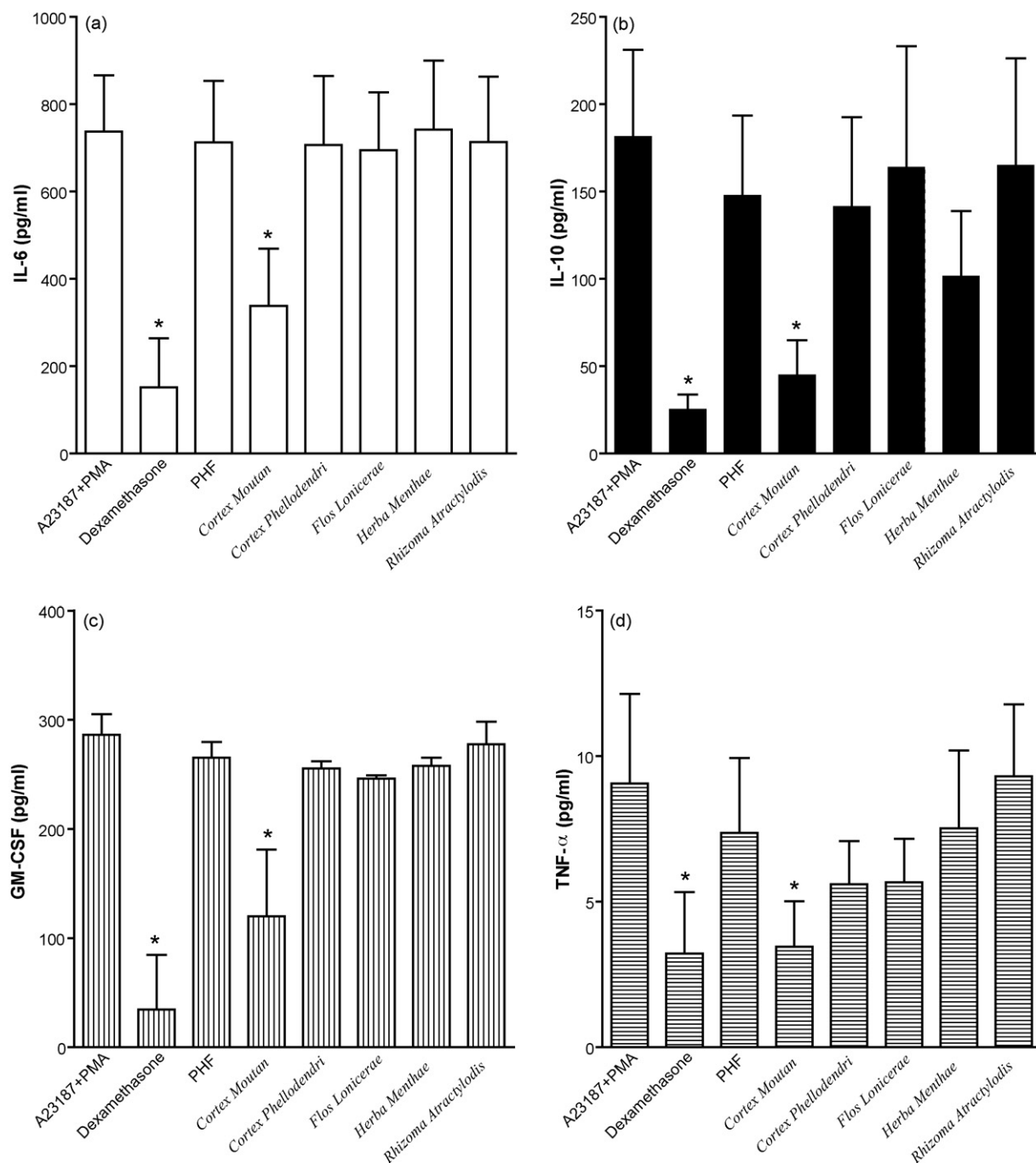


Fig. 3. Effects of PHF and individual components: *Cortex Moutan*, *Cortex Phellodendri*, *Flos Lonicerae*, *Herba Menthae* and *Rhizoma Atractylodis* on the synthesis of cytokines from HMC-1 incubated in a mixture of 1 μ M calcium ionophore A23187 and 50 nM phorbol-12 myristate 13-acetate (A23187 + PMA) for 24 h. The control levels of (a) IL-6, (b) IL-10, (c) GM-CSF, and (d) TNF- α produced by A23187 + PMA activated HMC-1 were 737.6 ± 128.5 pg/ml, 181.5 ± 49.65 pg/ml, 286.3 ± 18.9 pg/ml, and 9.1 ± 3.0 pg/ml, respectively. PHF and all herbs were tested at 500 μ g/ml and dexamethasone (1 μ M) was included as a positive control. Results are given as mean $\pm$ S.E.M. for $n = 4$. *Indicates $p < 0.05$ when compared with the control level of cytokine release induced by A23187 + PMA.

cells in AE patients. Since only *Cortex Moutan* (500 μ g/ml) but not PHF produced comparable levels of inhibition as dexamethasone on the production of all the tested cytokines, another reason for the discrepancy may be due to the presence of counteracting components which were rapidly metabolized or not absorbed when PHF was administered to patients. In this case, the steroid-sparing anti-inflammatory effects of PHF in our previous clinical trial (Hon et al., 2007b) would have been contributed mainly by the action of *Cortex Moutan*.

In conclusion, our current study has produced direct evidence that PHF can attenuate the release of preformed and lipid mediators from mast cells with preference for the peptidergic pathway which is activated by polybasic secretagogues such as substance P in skin mast cells. This mast cell stabilizing property of PHF is believed to contribute significantly to the clinical efficacy in the management of AE despite of the less conclusive results with HMC-1. Further studies with isolated primary human mast cells will provide the best support to this hypothesis. Based on the studies on individual

component herbs, we may anticipate from the results obtained in the current study that the clinical efficacy of PHF in relieving AE can be improved by increasing the ratio of *Cortex Moutan* and *Herba Menthae* or by decreasing the ratio of *Cortex Phellodendri*. Clinical investigations obviously are required to confirm such hypothesis. Meanwhile, the rat peritoneal mast cells can be a suitable in vitro model for evaluating the optimal ratios of the component herbs of PHF prior to testing the clinical efficacy of modified formulations.

Acknowledgments

We would like to thank Ms. Cheng L. and Mr. Lau C.P. for the herbal authentication works and Ms. Fong Y.S.C., manger of Centre for Clinical Trial on Chinese Medicine for her assistance towards the study as well as the supports of the Ming Lai Foundation and the International Association of Lions Clubs District 303- Hong Kong and Macau Tam Wah Ching Chinese Medicine Resource Centre.

References

- Alenius, H., Laouini, D., Woodward, A., Mizoguchi, E., Bhan, A.K., Castigli, E., Oettgen, H.C., Geha, R.S., 2002. Mast cells regulate IFN-gamma expression in the skin and circulating IgE levels in allergen-induced skin inflammation. *Journal of Allergy and Clinical Immunology* 109, 106–113.
- Aridor, M., Rajmilevich, G., Beaven, M.A., Sagi-Eisenberg, R., 1993. Activation of exocytosis by the heterotrimeric G protein Gi3. *Science* 262, 1569–1572.
- Chinese Pharmacopoeia Committee, 2005. *Chinese Pharmacopoeia*. Chemical Industry Press, Beijing.
- Emadi-Khiav, B., Mousli, M., Bronner, C., Landry, Y., 1995. Human and rat cutaneous mast cells: involvement of a G protein in the response to peptidergic stimuli. *European Journal of Pharmacology* 272, 97–102.
- Hon, K.L., Leung, T.F., Wong, Y., Lam, W.K., Guan, D.Q., Ma, K.C., Sung, Y.T., Fok, T.F., Leung, P.C., 2004. A pentaherbs capsule as a treatment option for atopic dermatitis in children: an open-labeled case series. *American Journal of Chinese Medicine* 32, 941–950.
- Hon, K.L., Lee, V.W., Leung, T.F., Lee, K.K., Chan, A.K., Fok, T.F., Leung, P.C., 2006. Corticosteroids are not present in a traditional Chinese medicine formulation for atopic dermatitis in children. *The Annals, Academy of Medicine, Singapore* 35, 759–763.
- Hon, K.L., Lam, M.C., Wong, K.Y., Leung, T.F., Ng, P.C., 2007a. Pathophysiology of nocturnal scratching in childhood atopic dermatitis: the role of brain-derived neurotrophic factor and substance P. *British Journal of Dermatology* 157, 922–925.
- Hon, K.L., Leung, T.F., Ng, P.C., Lam, M.C., Kam, W.Y., Wong, K.Y., Lee, K.C., Sung, Y.T., Cheng, K.F., Fok, T.F., Fung, K.P., Leung, P.C., 2007b. Efficacy and tolerability of a Chinese herbal medicine concoction for treatment of atopic dermatitis: a randomized, double-blind, placebo-controlled study. *British Journal of Dermatology* 157, 357–363.
- Leung, D.Y., 2000. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *Journal of Allergy and Clinical Immunology* 105, 860–876.
- Leung, R., Wong, G., Lau, J., Ho, A., Chan, J.K., Choy, D., Douglass, C., Lai, C.K., 1997. Prevalence of asthma and allergy in Hong Kong schoolchildren: an ISAAC study. *European Respiratory Journal* 10, 354–360.
- Leung, A.K., Hon, K.L., Robson, W.L., 2007. Atopic dermatitis. *Advances in Pediatrics* 54, 241–273.
- Leung, T.F., Wong, K.Y., Wong, C.K., Fung, K.P., Lam, C.W., Fok, T.F., Leung, P.C., Hon, K.L., 2008. In vitro and clinical immunomodulatory effects of a novel Pentaherbs concoction for atopic dermatitis. *British Journal of Dermatology* 158, 1216–1223.
- Lillie, T.H., Gomperts, B.D., 1992. Guanine nucleotide is essential and Ca²⁺ is a modulator in the exocytotic reaction of permeabilized rat mast cells. *Biochemical Journal* 288, 181–187.
- Mousli, M., Hugli, T.E., Landry, Y., Bronner, C., 1994. Peptidergic pathway in human skin and rat peritoneal mast cell activation. *Immunopharmacology* 27, 1–11.
- Shefler, I., Zavaro, O., Raz, T., Baram, D., Sagi-Eisenberg, R., 2008. Inhibition of basic secretagogue-induced signaling in mast cells by cell permeable G alpha i-derived peptides. *International Archives of Allergy and Immunology* 145, 131–140.
- Theoharides, T.C., Kempuraj, D., Tagen, M., Conti, P., Kalogeromitros, D., 2007. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunological Reviews* 217, 65–78.
- Wang, X.S., Lau, H.Y., 2006. Prostaglandin E potentiates the immunologically stimulated histamine release from human peripheral blood-derived mast cells through EP1/EP3 receptors. *Allergy* 61, 503–506.