Original Article

Corticosteroids are not Present in a Traditional Chinese Medicine Formulation for Atopic Dermatitis in Children

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Abstract

Introduction: Traditional Chinese medicine (TCM) has been used as an alternative in treating children with atopic dermatitis (AD) but its efficacy and potential side effects are debatable. We recently used a TCM capsule (PentaHerbs) on 9 children and observed significant reductions in clinical scores of disease severity. However, there have been concerns that the therapeutic effects of many forms of TCM are due to the presence of corticosteroids. The purpose of this study was to evaluate if common corticosteroids are present in PentaHerbs capsules. Materials and Methods: PentaHerbs powder was analysed with thin-layer chromatography, infra-red spectrophotometry and liquid chromatography mass spectrometry. Results: Hydrocortisone, prednisolone, fludrocortisone and dexamethasone were not detected in the PentaHerbs capsules. Conclusion: Corticosteroids are not present in the 5 familiar herbs that were earlier shown to have efficacy on AD.

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Key words: Atopic dermatitis, Corticosteroids, Traditional Chinese medicine

Introduction

Atopic dermatitis (AD) is a common, chronic inflammatory skin disease.[1] It has been estimated that 15% of schoolchildren aged 13 to 14 years have a history of AD.[2] Corticosteroids (CS), either in topical or systemic formulations, are the mainstay of treatment. CS have a wide range of immunomodulatory effects, including the suppression of cytokine production, adhesion molecule expression and leukocyte chemotaxis.[3] CS are also associated with deranged metabolism, growth suppression and increased susceptibility to infection. Potent topical CS can result in significant suppression of the hypothalamic-pituitary-adrenal axis.[4] Despite the use of CS and newer and more specific immunomodulatory agents, some patients remain recalcitrant and may seek complementary treatment in the form of Chinese herbs and traditional Chinese medicine (TCM). However, clinical trials on TCM in AD have been inconsistent[5-7] and the side effects of its use are worth concern.[7-13] In particular, there have been concerns that the therapeutic effects of TCM are due to CS.[11,14] We recently tested a TCM capsule (PentaHerbs capsule) in 9 children and observed significant reduction in clinical scores of disease severity with no apparent adverse effects.[15] The median (range) overall SCORAD score before and at the end of 3 months was 60.3 (20.0 to 82.6) and 40.0 (11.4 to 56.5), respectively ($P = 0.008$). Significant improvements were also noted in the components of the SCORAD index (extent, intensity, pruritus and sleep loss). This study aimed to evaluate if common CS are present in PentaHerbs capsules, using thin-layer chromatography (TLC), infra-red spectrophotometry (IRS) and liquid chromatography mass spectrometry (LC-MS).

Materials and Methods

The capsules had been manufactured, packaged and labelled by the Chinese Medicine Industry Development Centre, the Hong Kong Institute of Vocation Education, using the Good Manufacturing Practice (GMP) standard. The optimal composition of each herb included was standardised prior to manufacturing. The powder was formulated into uniform-dose capsules under the supervision of the Clinical Trials Section, Institute of Chinese Medicine

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of the Chinese University of Hong Kong according to established procedures. The original formula was: *Flos lonicerae* (*Jinyinhua* 金銀花) 2 grams, *Herba menthae* (*Bohe* 薄荷) 1 gram, *Cortex moutan* (*Danpi* 丹皮) 2 grams, *Rhizoma atractylodis* (*Cangzhu* 蒼朮) 2 grams and *Cortex phellodendri* (*Huangbaig* 黃柏) 2 grams (total, 9 grams of raw herbs daily). The extraction rate from this raw herb was around 18% to 20%, which made 6 to 7 capsules. The dose calculation was based on the assumption that patients had to be old enough to swallow the capsules without difficulty (about 7 years of age and above).[15] The TCM capsules were assessed by the Institute of Chinese Medicine for the presence of heavy metals, microbial products and residual pesticides to ensure that they met quality and safety requirements.

Thin-layer Chromatography (TLC)

The TLC system is a general screening method for steroid-based compounds.[16-18] Silica gel 60 F-254 0.2 mm 20 x 20 aluminium (Alltech Part no. 5539) was used as the plate and dichloroethane:methanol:water (95:5:0.2) for the mobile phase. Each drug was dissolved in 10 mL of methanol by sonication. The methanol mixture was filtered. The 4 corticosteroids and the PentaHerbs powder were applied on the plate using micropipettes, spotting 0.5 cm from the bottom of the plate. The samples were run in the TLC chamber and the solvent front was marked after completion and dried in air. The plate was then heated at 78°C after being sprayed with 50% sulfuric acid (H_2SO_4). The chromatogram was visualised under UV light. The retention factor (Rf) value, defined as the distance the substance travels from the origin divided by the distance the solvent front travels from the origin, was calculated.

Infra-red Spectrophotometry (IRS)

The TCM powder was examined by IRS at wavelengths of 250 to 4000 cm^{-1}, comparing with the spectrum obtained with prednisone. In this technique, the sample was dissolved in the minimum volume of methanol and methylene chloride mixture and evaporated to dryness on a water-bath. The mixture was then pressed into a flat disk and a small amount was ground and crushed with 1:100 potassium bromide (KBr). The Perkin Elmer Spectrum BX FT-IR (FT-IR system, Boston, USA) was used. Infrared analysis for structural identification has been facilitated by the construction of software for infrared spectral databases (Spectrum 2.00).

Mass Spectrometry (LC-MS)

The mass spectrometry procedure was carried out using the electrospray ionisation (ESI) method at positive mode. API 2000 Triple Quadrupole LC/MS/MS Mass Spectrometer (Perkin-Elmer Sciex Instruments, Boston, USA) was used. A build-in syringe pump (diameter 2.3 mm) produced a flow rate of 10 μL/min. Ammonium formate and methanol (with the ratio 1:1) were used as a buffer. The ESI source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets. The droplets become charged as they exit the capillary. As the solvent evaporates, the droplets disappear and leave highly charged analyte molecules.

Data are presented in the form of a graph of abundance against m/z. M/z represents the molecular weight of each of the molecular fragments and is expressed in atomic mass units (amu). The most abundant ion (the base peak) is usually assigned a relative abundance of 100% and all other ions are assigned values relative to the abundance of the base peak.

Results

The PentaHerbs formulation is a greenish powder packed within a dark green capsule. Each capsule contains 0.2677 g of dry powder. The powder readily dissolves in water, but is less soluble in methanol and ethanol. The PentaHerbs powder was compared with 4 known corticosteroids, namely hydrocortisone, prednisolone, fludrocortisone and dexamethasone (Table 1). The sulfuric acid reaction of steroids on TLC plates is illustrated in Table 2. The results show that the colour of the 4 types of CS matched the colour of the source.[16,17] However, the RF value of hydrocortisone, prednisolone, fludrocortisone or dexamethasone did not match those of the ingredients in PentaHerbs capsules.[16,17] IRS and LC-MS of the PentaHerbs were negative for CS, as illustrated in Figures 1 and 2.

Discussion

In a pilot study of PentaHerbs capsules in children, we observed significant reductions in clinical scores of disease severity and pruritus. There was also improvement in sleep and no apparent adverse effects.[15] This report confirms that the therapeutic and immunomodulatory effects of PentaHerbs capsules are not due to commonly used cortico-

Table 1. Four Corticosteroids were Used as Standard References Against PentaHerbs Capsules

Chemicals	Molecular weight	Label
Dexamethasone (4 mg)	392.5	D
Hydrocortisone (20 mg)	362.5	H
Fludrocortisone (100 ug)	380.5	F
Prednisolone (5 mg)	360.4	P
PentaHerbs capsule	335	U

Note: The molecular weights of prednisone, betamethasone, fludrocortisone acetate, and methylprednisolone are 358.4, 392.5, 422.5, 374.5, respectively.[19]

Table 2. Colour Changes and Rfs of the 4 Corticosteroids and PentaHerbs (Under 366 nm after Spraying with Sulfuric Acid)

Standard	D	H	F	P	U
Colour in UV (366 nm) after spray	No colour	Green	Bright spot	Orange Blue	Green
Rf value in UV (366 nm) after spray	3.66	4.87	14.63	7.31 2.44	2.43

Rf: retention factor

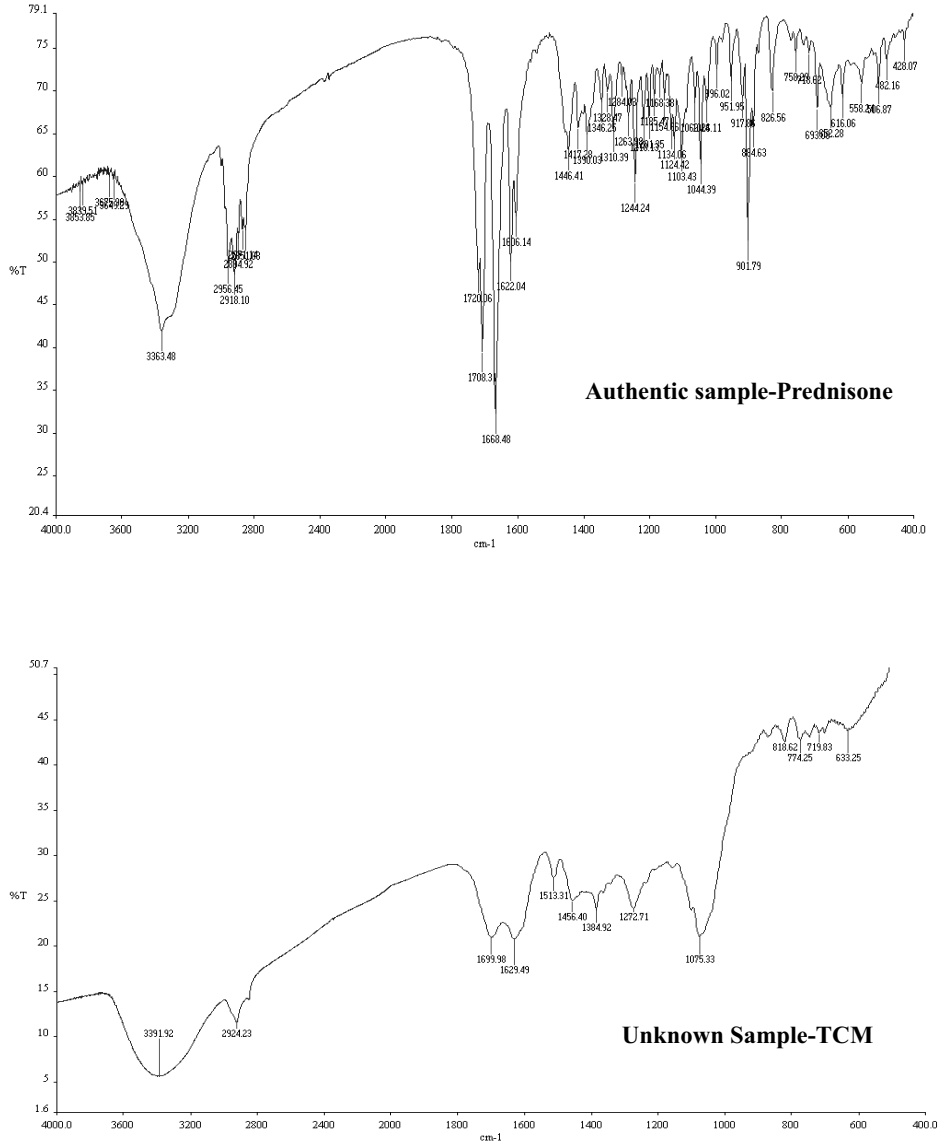

Fig. 1. Infra-red spectrum of prednisone and traditional Chinese medicine (TCM). Principal peaks at wavenumbers 1668, 1707, 904, 1622, 1610 and 1246 (KBr disk).

steroids, namely hydrocortisone, prednisolone, fludrocortisone or dexamethasone. *Flos lonicerae* (*Jinyinhua*) and *Cortex phellodendri* contain phytoestrogens (flavonoid and phellodendrine, respectively), which may be partially responsible for the anti-allergic effects of PentaHerbs. Both herbs are known to possess antibiotic, anti-inflammatory and immunomodulating effects.[20-23] The other 3 herbs are not noted for containing phytoestrogens. Herba menthae is well known for its menthol content, which is traditionally used against virus, pain and cough. *Cortex moutan* has been used for its anti-inflammatory and immunomodulating effects. It has also been used separately for allergic rhinitis. *Rhizoma atracytylodis* contains a minimal amount of phytoestrogen. It has been used for its anti-inflammatory effects.[20]

The 5 herbs constituting PentaHerbs capsules are commonly used components of Chinese medicine formulae and their details have been fully documented in the Chinese

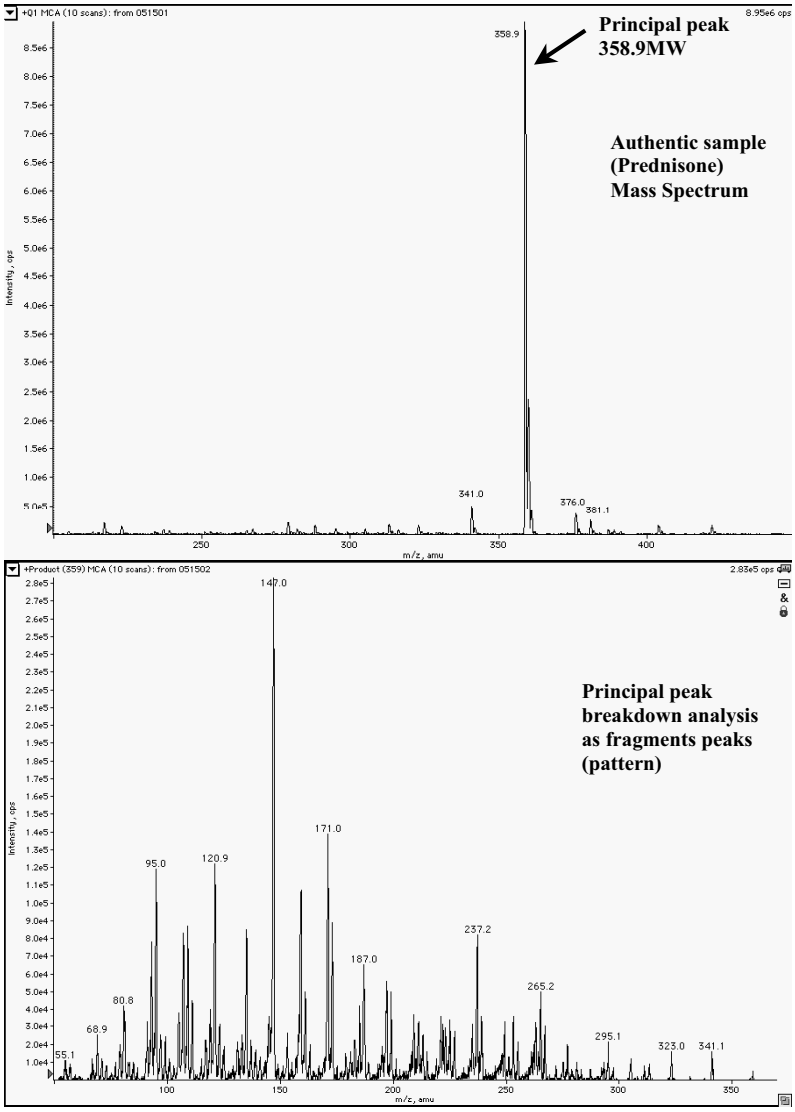

Fig. 2(a).

herbal dictionary.[20] The 5 herbs used in the study of this formulation were proposed to work according to the following TCM principles: *Jingyinhua* and *Bohe* clear damp-heat from the exterior, *Danpi* clears heat from blood, while *Cangzhu* and *Huangbai* clear damp-heat from the interior. These herbs have been extensively used in China for the treatment of allergic diseases including AD, asthma and allergic rhinitis. Pharmacological studies have documented that these herbs have anti-allergic, anti-inflammatory, anti-pruritic and sedative actions. They have been used in the study of childhood AD.[24,25] Based on the cited references and our results, we conclude that the efficacy of PentaHerbs on AD is not related to any therapeutic form of CS.

The successful studies noted earlier used *Clematis armandii*, *Lophatherum gracile*, *Dictamnus dasycarpus*, *Tribulus terrestris* and *Schizonepeta tenuifolia*, which have been shown to be beneficial for the treatment of severe AD.[5,6] In Hong Kong, a controlled trial of Zemaphyte (a standardised formulation containing 10 Chinese herbs) was conducted on 37 Chinese patients with recalcitrant AD for 8 weeks. No beneficial or adverse biochemical effects were demonstrated.[7] None of these herbs showed pharmacological evidence of anti-allergic and anti-inflammatory effects. Furthermore, *Clematis armandi*, used in Sheehan and Atherton's studies, has a diuretic effect and its long-term use may necessitate potassium supplementation to avoid hypokalaemia.[20] These herbs were not used in the manufacture of the PentaHerbs capsule in view of safety concerns.

Adverse effects of Chinese herbal medicines have been extensively reported.[7-13] In particular, there have been concerns that the therapeutic effects of various forms of TCM are due to the presence of CS.[11,14] This concern is heightened by the fact that TCM is not standardised and varies with the prescription of individual practitioners. It is believed that standardising herbal mixtures contradicts Chinese medical philosophy.[8]

The clinical effects of PentaHerbs capsules appear impressive in this trial, where we used standardised treatment with only one uniform formula of herbs.[15] Efficacy was clearly not due to any common CS. The 5 herbs are inexpensive and commonly used with other Chinese herbs in various Chinese formulae. Our results have added further reassurance in the use of these decoctions. More importantly, we have worked out the favourable proportion of the herbs in the formula. The capsular form prevents improper handling of herbs by the parents, and has made consumption simple. The exact mechanisms of action and therapeutic effects of PentaHerbs will be confirmed with further randomised and placebo-controlled clinical trials.

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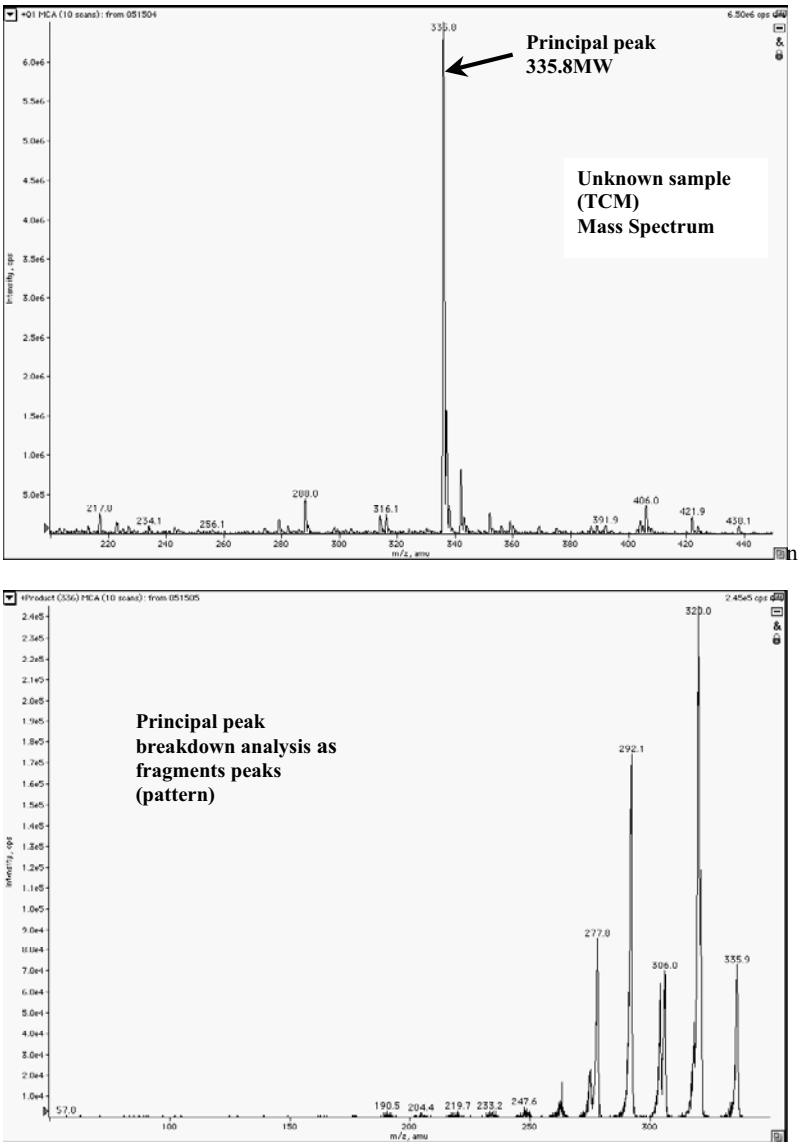

Fig. 2(b).

Fig. 2. Liquid chromatography mass spectrometry of (a) prednisone and (b) traditional Chinese medicine (TCM).

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