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Antiseptic, Antiperspirant and Deodorant Diaper for the Frail Older Adult with Urinary Incontinence

Keywords: Urinary incontinence; frail older adults; diaper; tradition Chinese medicine; sweat modulation

Abstract

Introduction: Urinary incontinence is a common complaint among older people which could cause great burden to the carer and healthcare system. The idea of creating a special diaper for the older and frail older people suffering from urinary incontinence to prevent complications arising from over-night and persistent wetting of the perineal region therefore appear appealing and beneficial.

Aim of the study: The aim of this s tudy was to perform further laboratory studies to understand the sweat modulation effect of the herbal formula, and to determine whether the herbal formula and the herbal formula containing diaper would cause any skin toxicity.

Materials and Methods: In vitro studies including the Acetylcholinesterase (AChE) activity assay, and measurement of chloride efflux by short circuit current assay was performed to understand the sweat modulation effect of the herbal formula. In vitro skin toxicity test was also performed to determine whether the herbal formula and the herbal formula containing diaper would cause any skin toxicity.

Results: Our herbal formula containing Cortex Fraxini, Mori Follium and Calamine significantly increased the acetylcholinesterase activity in PC12 cells. This formula also significantly inhibited the UTP-evoked CI- secretion and ACh-induced CI- transport. Further skin toxicity test suggested this formula exerted no significant toxicity to the cells in the skin.

Conclusion: These data further supported the positive data observed in two clinical studies previously completed which demonstrated that the herbal formula could reduce sweat secretion, reduce odour, skin irritations and the herbal extract-containing diaper could improve the quality of life of individuals with urinary incontinence.

Introduction

Urinary Incontinence (UI) is a common complaint among the elderly, particularly in the frail older adults [1]. Population-based studies suggested that UI is more common in women than men. Prevalence of UI also increases with age, with more than 40 % of the female population affected at the age of 70 years and above. With the global rapidly aging population, UI is expected to cause a heavy burden to the household communities and the healthcare system worldwide [2]. Due to the involuntary urinary leakage, UI could cause discomfort to the individuals, thus acquiring much labour attention and assistance in the tedious apparel care. Prolonged exposure of urine to the skin could also further lead to perineal dermatitis or infections as a result of poor hygiene and microbial infections. These skin injuries would then further lead to skin abrasions and ulcerations [3,4]. Furthermore, the bacterial and fungal overgrowth within the diapers could also cause unpleasant foul odours [4-6]. The idea

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of creating a special diaper for the frail older adults suffering from urinary incontinence to prevent complications arising from overnight and persistent wetting of the perineal region is thus appealing. Previously, we had reported the creation of an absorbent, antiseptic, antiperspirant and deodourant diaper containing traditional Chinese herbs, Mori Follium, Fraxini Cortex and Calamine. Mori Follium and Fraxini Cortex are herbs traditionally used in oral concoction as antiperspirant, whereas Calamine is a traditional Chinese medicinal herb commonly used topically as antipruritics. These three herbs, mixed in the ratio 1:1:1 were integrated into the diaper, and tested in two different clinical trials. The first of which utilized a convenient sweating assessment machine, Sudoscan for evaluation of sweat gland function. Sudoscan acts by measuring the ability of the sweat glands to release chloride ions (i.e. chloride conductance in microsiemens μS) in response to an electrical stimulus on the palms and soles (areas with the highest sweat gland density) as a biomarker to assess sweat gland function [7,8]. In the clinical study, volunteers were allowed to soak their feet inside herbal extracts water bath [7,9,10]. Sweat gland activities of the soles of the healthy volunteers were then assessed before and after the bath. It was reported that 84.6 % of the volunteers experienced a 15.3 % sweat reduction after the 15-minute herbal bath. The second pilot clinical trial was conducted in the dependent or disabled elderly using the innovative herbal extract impregnated diapers [11]. The dependent or disabled elderly were required to wear the herbal extract containing diapers in their Rehabilitation Home overnight continuously for six hours, for 5 consecutive days. Feedback and questionnaires were acquired from these dependent elderly and their carers, and results were compared with their feedback of wearing the control diaper alone without the

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herbal extract. Comparing with their control diapers, the majority of volunteers preferred the herbal extract containing diapers due to the less leakage and unpleasant odour. They were also found to have better skin conditions [4]. These clinical studies demonstrated that our herbal extract containing diapers could reduce sweat production and improve the quality of life of the elderly people.

Here, we have reported further laboratory studies to understand the underlying mechanism contributing to the observed beneficial effects of the herbal extract in previous clinical studies. We conducted several *in vitro* studies including the Acetylcholinesterase (AChE) activity assay to understand the anti-cholinergic effects of the herbal extract; and measurement of chloride efflux by short circuit current assay to understand the sweat modulation mechanism of the herbal extract. *In vitro* skin toxicity test was also performed to determine whether the herbal extract and the herbal extract containing diaper would cause any skin toxicity.

Materials and Method

Herbal materials authentication and preparation

Herbal material authentication: Raw herbal material of Mori Follium and Fraxini Cortex were purchased from a renowned supplier in Hong Kong. Calamine powder (Pharm Grade) were purchased from Wing Hing Chemical Co. Ltd., Hong Kong. Mori Follium and Fraxini were chemically authenticated using Thin Layer Chromatography (TLC) in accordance to Chinese Pharmacopoeia (CP) [12]. Upon chemical authentications, herbarium voucher specimen of Cortex Fraxini and Folium Mori were deposited at the museum of the Institute of Chinese Medicine at the Chinese University of Hong Kong, with voucher specimen number as 2016-3491 and 2016-3490, respectively.

Herbal extract preparation

Extraction of Cortex Fraxini and Folium Mori were performed at the Hong Kong Institute of Biotechnology (HKIB) following the traditional practice of herbal extraction in accordance to Good Manufacturing Practice (GMP). Briefly, raw herb was extracted twice by heating under reflux at 100 °C using 10x distilled water for each extraction. The aqueous extracts were combined and filtered. Filtrate was concentrated under reduced pressure at 60 °C. The concentrated extract was then spray dried with no excipient added. The dried extracts were packed in vacuum condition and stored until use.

In vitro cell culture experiments

Cell culture: The PC12 cell line was obtained from the American Type Culture Collection (USA). Cells were grown and maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10 % (v/v) horse serum (Gibco, USA), 5 % (v/v) fetal bovine serum (Gibco, USA), 100 U/ml penicillin, and 100 μ g/ml streptomycin in a 10 % CO₂ humidified atmosphere at 37 °C. Cells grown to 80 % confluence in T75 culture flasks were trypsinized and seeded into 6-well culture plates for experiment.

Inhibition of chloride secretion using the short Circuit current assay was performed using NCI-SG3 cells, the human eccrine sweat gland epithelial cell line. The cell line was kindly provided by Prof. Ko Wing Hung, School of Biomedical Sciences, the Chinese University

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of Hong Kong. NCL-SG3 cells were maintained in Williams Medium E, supplemented with epidermal growth factor (0.1 %), fetal bovine serum (5 %), Glutamic monosodium salt hydrate (2 mM), hydrocortisone (0.01 %), ITS-liquid supplement (1 %) and penicillin (1 %). Cultures were maintained at 37 °C in a humidified atmosphere of 5 % CO₂.

Acetylcholinesterase activity assay

Acetylcholinesterase assay was performed following the manufacturer's protocol. Briefly, 6-well plate was coated with 0.1 mg/ ml Poly-L-Lysine overnight. PC12 cells were seeded at 4 x 10⁵ cells/ well. Cells were then treated with various concentrations of herbal extracts (0 - 1 mg/ml) for 48 hrs. Cells were sonicated with RIPA buffer on ice for 30 minutes (Sigma-Aldrich, St. Louis, MO. USA.), followed by centrifugation at 14,000 x g. Cell lysate were mixed with the reaction mix solution containing acetylthiocholine stock solution, followed by incubation at 37 °C for 1 hr, and absorbance was measured spectrophotometrically at 410 nm.

Short Circuit current assay

Membranes response to Cl- transportation was monitored with Ussing chambers as previously described [13]. Prior to the experiment, NCL-SG3 cells (3 x 105/ 250 µL) were grown on MFTM Membrane Filters for 7 days. Fresh medium was changed every other day to obtain confluent cell monolayer. The integrated monolayer was mounted between two halves of Ussing chambers, which were filled with 7 ml of Krebs- Henseleit Solution (KHS) on both sides. The K-H solution contained the following components: 25mM NaHCO₂, 117 mM NaCl; 4.7 mM KCl; 2.5 mM CaCl₂; 1.2 mM KH₂PO₄; 1.2 mM MgSO $_4$; and 11 mM D-glucose; its pH was 7.4 when bubbled with 5% CO₂/95% O₂ and was kept at 37 °C by water jacket before use. Inhibition of chloride secretion on NCI-SG3 was then analyzed. Once the condition had stabilized (20-30 min), pre-incubation of herbal formula (at 1,2 or 3 mg/ml) or KHS (as blank control) on the basolateral side was performed for 10 min. Effect of herbal formula on efflux of Cl⁻ ion induced by UTP or ACh were then investigated by adding 10 μ M of UTP or ACh and incubation of 10 min. The short-circuit current was recorded by a short-circuit clamp amplifier and displayed using a chart recorder. And the current inhibited by herbal formula was calculated using the resultant change of current resistance.

In vitro skin toxicity test

Epiderm culture

A commercially available human epidermal equivalent, EpiDerm (EPI-200, MatTek Corporation, Ashland, MA, USA), was used as an *in vitro* model of the epidermis in this study since the general morphology of this model system mimicked that of normal human epidermis. These EpiDerm cultures comprised of human-derived epidermal keratinocytes, which were cultured on standing cell culture inserts (Millipore, Billerica, MA, USA) at the air-liquid interface to form a multilayered, differentiated model of the human epidermis. The *in vitro* skin toxicity test was performed as described previously in our laboratory [14,15]. Upon kit arrival, the EpiDerm cultures were placed in 6-well plates, and pre-conditioned overnight at 37 °C and 5 % CO₂. To determine if the different herbal formula (with or without

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were expressed as means \pm SD, n = 4-7 per group.

diaper) had toxic effects on skin, samples together with positive and negative controls were tested in the following experiments. 22 mg of the samples were added directly to the inserts. Diapers loaded with herbal extract were cut according to the size of the skin insert (0.63 cm²) and used as patches for the experiment. 5% SDS (supplied by the manufacturer of MatTek Corporation) was used as the positive control, with non-treated skin insert served as the negative control.

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MTT assay-cell viability test

Skin inserts were transferred to fresh plates with pre-filled MTT solution, and allowed to incubate for 3 hrs at 37 °C and 5 % CO_2 . Upon completion of incubation, all MTT solution was removed. Skin inserts were transferred to fresh plates and isopropanol was added to each inserts for 2 hrs for formazan extraction, before transferring to 96-well plate for spectrophotometric analysis at 550 nm. Cytotoxicity was expressed as the ratio of the cell viability, per treatment, to the maximum cell viability from the negative control (non-treated skin insert).

Statistical analysis

Data were presented as means \pm SD for all *in vitro* experiments, and means \pm SEM for all *in vivo* experiments. Prism 5 for Window (version 5.0c, GraphPad Software, Inc., USA) was used for statistical analysis. Significant differences among all groups were assessed by one-way ANOVA, followed by Bonferroni's Multiple Comparison Test. A probability of p < 0.05 was considered to be statistically significant.

Results

In vitro cell culture experiments

Acetylcholinesterase activity assay: To determine the anticholinergic effect of the herbal formula, acetylcholinesterase assay was performed to understand whether our herbal formula could enhance acetylcholinesterase activity to reduce acetylcholine levels, thereby reducing sweat stimulation. Figure 1 showed the effects of the herbal formula or individual Chinese herb on Acetylcholinesterase (AChE) activity. Individual Chinese herb increased AChE activity compared to control group in a dose-dependent manner. When being formed as a formula, the herbal formulae also had a trend to increase AChE activity at the higher dose of 1 mg/ml, suggesting that the herbal formula could enhance acetylcholinesterase activity to reduce acetylcholine in order to exert inhibitory effect on sweat control.

Short Circuit current assay

In the short circuit current assay, UTP and Acetylcholine (ACh) were used to stimulate chloride ion transport, and the effects of the herbal formula on chloride ion transport were tested under both inductions. Figure 2 showed a schematic diagram of the apparatus. The apparatus will generate a chart as recorded by the short-circuit clamp amplifier, and data were presented as percentage relative to UTP- or ACh-induced current under normal control condition without any treatment. For UTP-induced Cl⁻ secretion, herbal formula significantly inhibited the UTP-evoked Cl⁻ secretion at 1 mg/ml and 3 mg/ml Figure 3. Similarly, ACh-induced Cl⁻ transport was also inhibited by the herbal formula extract Figure 4. These data



Figure 2: (a) Schematic diagram of the Ussing chamber; (b) chart recorded by the short-circuit clamp amplifier for the short circuit current assay

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Figure 3: Effect of the herbal formula on UTP stimulated increase on CI- ion transport across NCI-SG3 monolayer (n = 3 - 4). Values represent mean \pm S D. UTP stimulated increase in short-circuit current, application of herbal formula inhibited these pulses.

suggested that the herbal formula extract could possibly regulate via both the purigenic and cholinergic signalling.

In vitro skin toxicity test

There was no significant effect exerted by the herbal formula extract (with or without diaper) on the skin inserts. There was also no significant toxicity effect of any of the individual Chinese herb on the skin inserts tested, suggesting the herbal formula extract, with or without diaper and its individual Chinese herbs are safe to use on human skin Figure 5.

Discussion

In the present project, we demonstrated the herbal formula containing Cortex Fraxini, Mori Follium, and Calamine significantly increased the acetylcholinesterase activity in PC12 cells. This formula also significantly inhibited the UTP-evoked Cl⁻ secretion and ACh-induced Cl⁻ transport. Further skin toxicity test suggested this formula exerted no significant toxicity to the cells in the skin. These data further supported the positive data observed in our two previous clinical studies which demonstrated that the herbal formula could reduce sweat secretion and the herbal extract-containing diaper could improve the quality of life of individuals with Urinary Incontinence (UI).

Urinary incontinence is a symptom that is commonly seen among the elderly and people suffering from paraplegia. It has been estimated that up to 40 % people over 65 years of age living in their own accommodations suffer from incontinence, with those living in the nursing homes having an even higher prevalence [1]. Clinically, UI can be classified into urge UI (UUI) and Stress UI (SUI), based on their pathophysiology and causal factors [16]. Myogenic or neurogenic are the common casual factors contributing to UUI. SUI on the other hand, is due to abdominal pressure such as sneezing, coughing, exercise, lifting, or position change etc. Treatment method for UI depends on the types of UI and their symptoms [16]. Oxybutynin, which is an anti-cholinergic drugs that is capable of blocking the action of the neurotransmitter acetylcholine is a widely used treatment option for UUI [16,17]. It is often used in combination with other behavioural changes therapy such as pelvic floor muscle training exercises [18]. Nevertheless, caution regarding the use of it is raised, particularly in frail elderly patients due to the



Figure 4: Effect of the herbal formula on ACh stimulated increase on Cl- ion transport across NCI-SG3 monolayer (n = 3 - 4). Values represent mean \pm S D. Ach stimulated increase in short-circuit current, application of herbal formula inhibited these pulses.



Figure 5: Effect of different samples on skin toxicity. Values were expressed as means \pm SD, n = 4 per group. Significant difference between PBS control and SDS control: *** p < 0.001 using Student's t-test. Significant difference between PBS control and individual herbs or all formulations: ## p < 0.01 using one-way ANOVA, followed by Bonferroni's Multiple Comparison Test.

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increased risk of cognitive impairment [19, 20]. It is also not ideal nor practical for the frail elderly to have pelvic floor muscle training as their treatment option. The ability of the herbal extract containing diaper to improve the quality of life of the frail elderly in our clinical studies, which is associated with anti-cholinergic activities in further laboratory experiments, is therefore appealing.

In designing the diaper for these frail elderly, it is important to note that clinically, sweating is a common phenomenon observed in frail elderlies acquiring diapers and the ability of the herbal extract containing diaper to control sweat modulation would be of an added value. In previous clinical study using the sudoscan, we observed that our herbal extract is capable of reducing sweat production. Further mechanistic studies in the laboratory using short circuit assay suggested that our herbal formula extract could inhibit Cl⁻ secretion and transport induced by ACh and ATP, thereby supporting the concept that our herbal extract is capable of reducing sweat secretion induced by both cholinergic and purinergic regulation.

Urinary incontinence not only affects the quality of life of the sufferers, but it could also lead to dermatitis dependency, sleep disturbances as well as downgrading the person's dignity, mood, and quality of life [21]. In today's busy and possibly crowded settings of the elders' home, incontinence tends to significantly affect the environmental hygiene and careers' moral [5]. Furthermore, frail older persons often suffer from co-existing illnesses and comorbidities which require intensive assistance from health care staff, thereby further increasing the burden of the carers. With such adverse reality, the idea of creating the rehabilitation devices such as diaper which are highly absorbent, and could potentially prevent infection to improve the quality of life of the old people with incontinence to relieve the workload of the carers would be very much appreciated. Here, our laboratory has provided pilot clinical studies supporting the efficacy of this Chinese herbal extract containing diaper, which is further supported by bench experiments not only on the mechanisms behind but also on its safety as a topical agent. The encouraging results had provided support for this novel herbal material containing diaper to be developed as a common rehabilitation device for marketing in the near future.

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