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Can herbal extracts be used as skin penetrating agent

Leung Ping Chung[1,2,*], Zhao Xin[1,2], Law Wai Tak[1] and Lau Tai Wai David[1]

[1]*Institute of Chinese Medicine, The Chinese University of Hong Kong, School of Public Health, Prince of Wales Hospital, Shatin, NT, Hong Kong;* [2]*Department of Orthopaedics and Traumatology, Prince of Wales Hospital, Shatin, Hong Kong*

SUMMARY

Swollen and painful extremities have been treated with herbal baths in attempts to alleviate the symptoms. A popular herbal bath used in China, contains a component called 'bone-penetrating herb' which is believed to facilitate the penetration of herbal substances across the skin to the swollen site, so that pain and swelling will be improved. A search from the Chinese pharmacopedia revealed that 22 different herbs have been traditionally used as 'bone-penetrating herb'. Five of these herbs were available in market and were chosen for experimental studies. Standard diffusion experiments were done to identify the most effective herb among the five, in the penetration facilitation. Glechoma longituba at a concentration of 20% was found to give the best results in the facilitation of Bromophenol blue diffusion across artificial and biological membranes. When compared with one commonly used diffusion facilitator, viz. azone, azone was found to be more effective than glechoma longituba. The encouraging observations support future studies on the basic science behind the use of herbal components as topical agents to treat pain and swelling.

Key words: Herbal treatment; Diffusion; Topical agent; Rehabilitation

INTRODUCTION

Administration of pharmaceuticals via the skin is popular, not only in the field of dermatology, but in other areas like swellings and pain of the extremities where frequent therapy, in the form of topical baths, is given. (Jia *et al*., 1991; Wang, 2002; Ji *et al*., 2004).

The use of herbal medicine, either singly or in combination, as topical agents, in the form of a paste or a bath, has been traditionally popular for hundreds of years in the practice of Chinese Medicine. Topical application of Chinese Medicine is particularly popular for pathologies related to the musculo-skeletal system, often associated with swellings, inflammation and pain. (Skelly and Shah, 1987; Gu and Bao, 2000; Chang and Ye, 2005) Topical applications, under such circumstances, assume that effective components of the herbs penetrate through the skin barrier and become distributed to the underlying tissues. The local effects produced are dependent on the direct diffusion, while any general, systemic effects follow subsequent absorption and circulatory distribution.

Some herbs, traditionally used for such purposes, carry the name of "Bone-penetrating herb" which implies that these herbs are capable of per-cutaneous transport. These herbs could better be understood as skin penetrating agents. (Jia *et al*., 1991; Tang *et al*., 2002).

*Correspondence: Leung Ping Chung, Institute of Chinese Medicine, The Chinese University of Hong Kong, School of Public Health, Prince of Wales Hospital, Shatin, NT, Hong Kong. Tel: +85222528872; Fax: +85226325441; E-mail: pingcleung@cuhk.edu.hk

In this study, we evaluated a number of these "Bone-penetrating herbs" to see whether they could facilitate skin transport.

MATERIALS AND METHODS

Choice of herbs

One herbal broth containing 13 herbs has been a popular topical agent in China, used for the treatment of swollen, painful hands, caused by injury or other pathologies. Patients soak their affected hands into the warm herbal bath on regular intervals (usually daily or twice daily) to control of the swelling and pain. The herbal formula contains one 'Bone Penetrating Herb', which is assumed to be crucial for the effectiveness of the bath. (Gu and Bao, 2000) When the Chinese Pharmacopoeia, was searched for 'bone penetrating herb', 22 varieties were found. Each variety had its unique origin of growth and different prescribers used different species carrying the same common name of 'bone-penetrating herb'. Five of the better known species were chosen as the targets of our investigation (Chang and Ye, 2005).

The Botanical names of the five "Bone-penetrating herbs" are as follows:

- Speranskiae tuberculata (Bunge) Baill
- Sambucus williamsii Hamce
- Glechoma longituba (Nakai) Kupr
- Vicia amoena Fisch
- Cynanchum paniculatum (Bunge) Kitag

All the five herbs are general items included in the complex herbal formulae used for treating fractures. The expected effects include pain relief, swelling control and fracture healing. The experimental design was an *in-vitro* study using standard experimental diffusion devices (Fig. 1) with donor and recipient chambers.

Diffusion membranes used, included an artificial nylon filter membrane, and two biological membrances, viz porcine peritoneal membrane and porcine split skin. Using filter paper and biological membranes to test the diffusion enhancement effects of pharmaceutical agents is a standard essential step in the exploration of the skin penetrating property of topical chemical agents (Brain *et al.*, 2002; Tang *et al.*, 2002).

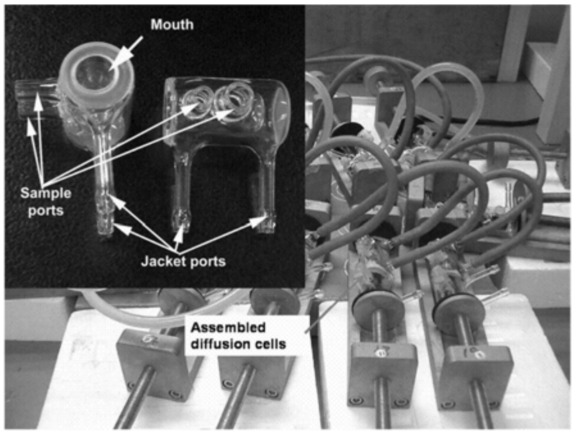

Fig. 1. A photograph showing the structure of the components of diffusion cells and when they were assembled.

Solution

Phosphate buffer saline (PBS) was used as the solution. On the donor side 0.5% Bromophenol blue (BPB) was added. The dye BPB was diffusing through the mounted membrane and its speed of diffusion would be affected by a facilitating agent, i.e. the herbal extract.

Facilitating agent

Extracts of the five different types of "bone penetrating herb" was used separately in the diffusion chambers.

The herbs were purchased from the Mayway (Anguo) Chinese Medicinal Herbs Co. LTD (China). Extracts were made by standard boilding and reflux method, details are given as follows:

The raw herb was weighed and put into the boiling container. 500 ml of distilled water was added. Boiling was commenced and continued for 2 h. The decoction was collected, and equal volume of fresh distilled water was added for a second boiling which lasted the same duration. After filtering the two decoctions, the solution was condensed via a vacuum evaporator. The condensed decoction was lyophilized and the powder was collected, weighed, bottled and kept inside a dessiccator ready for use. The weight of the powder extracted from one gram of raw herb could be easily calculated.

Diffusion was tested with a standard control and later, a positive control using 1% water soluble of azone. Azone was the most commonly used diffusion facilitator in medicinal ointments.

Experimental procedures

Donor cells were filled with 20% herbal extract solutions of Phosphate buffer saline (PBS), with 0.5% Bromophenol blue (BPB). The receptor cells contained just PBS. The cells were stirred with magnetic fleas at 37°C.

At specific intervals, samples were taken out form the recipient cell and replaced with fresh PBS of the same volume. The amount of BPB diffused across the membrane was estimated by ultraviolet spectrophotometry, calculated according to the standard concentration curve of BPB.

The accumulative amounts of the penetrated permeant i.e. BPB, were plotted as a function of time, and the steady-state flux was calculated using regression with SPSS software package.

Three main sets of experiments were performed:

I. To identify the herb with the most effective penetrating property, i.e., the herb that best facilitates the diffusion of BPB.

II. To investigate the effectiveness of the most effective herb on the diffusion through 2 different types of membrane, using different concentrations.

III. To compare the penetrating effect of the most effective herb with a well known, commercial agent used as percutaneous penetrator – azone, using a skin membrane.

RESULTS

Identification of the herb with the most effective penetrating property

A. Using an artificial filter paper

Five "bone-penetrating herbs" which had been separately made into extracts were tested. A standard nylon filter paper was used as the diffusion membrane. Using the standard procedures described, four of the five herbs, at concentrations of 20% (the extracts of 20 g raw herb/100 ml PBS) were found to enhance the penetration of BPB, compared with the negative control ($P < 0.005$). Of the four herbs, Glechoma longituba gave the most effective enhancement of penetration (Table 1).

B. Using a biological filter membrane

The same experiments were repeated, using instead,

Table 1. Steady-state fluxes and the enhancement factors of BPB through nylon filter paper resulting from the application of five different "Bone-penetrating herbs"

Group	Steady-state flux J (μg/cm^2/h)		Enhancement factor (Foot note)
	Average	SD	
0.01% BPB control (n = 13)	10.81*	1.85	-
20% *Speranskia Tuberculata* (n = 9)	8.38*	2.61	0.8
20% *Sambucus Williamsii* (n = 9)	18.85*	2.01	1.7
20% *Glechoma Longituba* (n = 9)	23.23*	2.19	2.1
20% *Vicia Amoena* (n = 9)	17.21*	1.74	1.6
20% *Cynanchum Paniculatum* (n = 9)	15.07*	0.98	1.4

*Steady-state flux J was compared with the Steady-state flux *J* of control group by Mann-Whitney U test (two-tailed), the difference was significant at the 0.05 level. Enchancement factor (EF) is the ratio of the mean of the steady state flux (J) of the group treated with herbs to the steady state of flux J of the control group, and is given as EF = J1/J0, J1 = test value, J0 = Control value

Table 2. Steady-state fluxes and the enhancement factors of BPB through porcine parietal peritoneal membrane resulting from the application of five different "Bone-penetrating herbs"

Group	Steady-state flux *J* (μg/cm^2/h)		Enhancement factor
	Average	SD	
0.01% BPB control (n = 9)	3.39	2.74	-
20 % *Speranskia Tuberculata* (n = 5)	5.07	0.88	1.5
20% *Sambucus williamsii* (n = 6)	10.63*	2.19	3.1
20% *Glechoma longituba* (n = 6)	10.92*	1.88	3.2
20% *Vicia amoena* (n = 5)	5.84	1.21	1.7
20% *Cynanchum paniculatum* (n = 5)	6.23	1.27	1.8

*Steady-state flux J was compared with the Steady-state flux *J* of control group by Mann-Whitney U test (two-tailed), the difference was significant at the 0.05 level.

Table 3. Steady-state fluxes and the enhancement factors of bromophenol blue through nylon filter paper, using five different concentrations of Glechoma longituba

Group	Steady-state flux *J* (μg/cm^2/h)		Enhancement factor
	Average	SD	
0.01% BPB control (n = 10)	9.35	2.22	-
1 % *Glechoma longituba* (n = 10)	15.57*	1.64	1.7
5% *Glechoma longituba* (n = 10)	17.59*	1.82	1.9
10% *Glechoma longituba* (n = 10)	17.86*	1.92	1.9
20% *Glechoma longituba* (n = 10)	18.64*	0.96	2.0
40% *Glechoma longituba* (n = 10)	17.72*	2.29	1.9

*Steady-state flux J was compared to the Steady-state flux *J* of control group by Mann-Whitney U test (two-tailed), the difference was significant at the 0.05 level.

a biological filter membrane, viz, porcine parietal peritoneal membrane. This membrane was easily prepared by pealing off the parietal peritoneal membrane from the abdominal cavity of a newly slaughtered pig.

The results of the experiments followed closely those just described, using the nylon filter paper. (Table 2) Of the five herbs which all showed facilitating effects on the diffusion of BPB, glechoma longituba gave the best results ($P < 0.005$).

Effectiveness of the most effective herb on the diffusion through 2 different types of membrane, using different concentrations

The experiments were repeated using different concentrations of Glechoma longituba in chambers of nylon filter paper and porcine peritoneum. 20% concentration was found to be the best concentration for penetration enhancement in the filter paper model (Table 3) but 10% worked best in the peritoneal membrane model (Table 4). The difference was not unexpected because diffusion through a biologically viable membrane was different from a dead, artificial membrane.

Comparison of the penetrating effects of the most effective herb with a well known, commercial agent used as percutaneous penetrator – azone, using a skin membrane

Glechoma longituba at concentration of 20% was tested against water soluble Azone in the penetration using Bromophenol blue (BPB), through the Biological membrane of porcine split skin. The split skin was easily prepared by applying boiling water to the skin of a freshly killed pig and then pealing off the surface layer. Table 5 shows the results of the

Table 4. Steady-state fluxes and the enhancement factors of bromophenol blue through porcine parietal peritoneal membrane, using four different concentrations of *Glechoma longituba*

Group	Steady-state flux *J* (μg/cm²/h)		Enhancement factor
	Average	SD	
0.01% BPB control (n = 15)	3.10	2.56	-
5% *Glechoma longituba* (n = 13)	8.32*	1.70	2.7
10% *Glechoma longituba* (n = 13)	8.58*	2.36	2.8
20% *Glechoma longituba* (n = 13)	8.11*	1.59	2.6
30% *Glechoma longituba* (n = 14)	7.92*	1.32	2.6

*Steady-state flux J was compared with the Steady-state flux *J* of control group by Mann-Whitney U test (two-tailed), the difference was significant at the 0.05 level.

Table 5. Steady-state fluxes and the enhancement factors of bromophenol blue through porcine epidermal membrane, using Glechoma longituba or water soluble Azone for enhancement

Group	Steady-state flux *J* (μg/cm²/h)		Enhancement factor
	Average	SD	
0.5% Bromophenol control (n = 8)	143.0	61.50	-
5%*Bromophenol control* + 1% *Water soluble Azone* (n = 8)	335.44*	89.23	2.3
5%*Bromophenol control* + 1% *Glechoma longituba* (n = 8)	240.83*	75.89	1.7

*Steady-state flux J was compared with the Steady-state flux *J* of control group by Mann-Whitney U test (two-tailed), the difference was significant at the 0.05 level.

penetration enhancement of the Glecoma longituba and azone. Azone was found to be more effective in the facilitation of diffusion of BPB.

DISCUSSION

The primary objective of this study was to investigate the possible facilitating effects of some herbs known as ‘bone penetrating herb’, on the transdermal diffusion of chemicals. To do so, a detectable chemical, viz Bromophenol blue, was selected and a convenient, validated method to quantitatively distinguish it in the receptor solution after diffusion through a standard membrane, was established (Chang and Ye, 2005). Different herbal extracts were used to facilitate the Bromophenol blue penetration.

Our experiments indicated that all 5 herbs did show different enhancement effects on the diffusion of Bromophenol blue across the membranes. The herbs showed different enhancing powers and Glechoma longituba was the strongest of all. The five herbs we used belonged to different botanical families and expectedly, the chemical components that could be affecting diffusion must be widely different. We could not offer any explanation to our observation that Glechoma longituba slowed the best facilitating effects on membrane diffusion. If we could get hold of all 22 ‘bone-penetrating herbs’ and subject all of them to the diffusion experiments, some other plant might demonstrate ever-more positive results.

Different concentrations also showed different enhancing powers and a concentration of 20 g raw material per 100 ml solution i.e. 20% concentration was observed to give the best enhancing effects across the filter paper membrane, although the differences between different concentrations were not remarkable. When a different (biological) membrane was used, the best concentration changed. While different diffusion behaviors is expected with different membranes, our observation reminded us that what we observed under laboratory conditions, should not be assumed to be identical with the clinical situations.

The overall results suggested that the herbs we used did show facilitatory effects on the membrane penetration of a high molecular substance i.e. Bromophenol blue. Whether the complex organic molecules contained in herbal decoctions would be similarly facilitated in a diffusion transfer would need to be tested in future studies. When compared with a popular commercial agent used for penetration enhancement, viz azone, azone was found to be more effective. Whether azone, used together with 'bone-penetrating herb', would give even better effects, would deserve exploration.

While agents like ethanol and azone have been used as facilitatory agents to enhance the penetration of skin when medicinal materials are applied topically, some herbal extracts might possess the similar properties. Such herbal extracts might be included in the preparation of topical agents (Andega *et al.*, 2001; Brain *et al.*, 2002). After all, folk-practice like herbal baths and rubs, primitive they might appear, might involve complicated processes of diffusion, absorption and interaction. And such traditional practices might have already included special herbs that possessed the enhancing properties on diffusion. As a continuation of this study, we have started to test for diffusion enhancement using a combination of herbs. Preliminary results showed additional effects which would be reported in future.

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