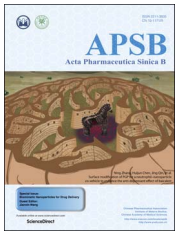
Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Synergistic immunoreaction of acupuncture-like dissolving microneedles containing thymopentin at acupoints in immune-suppressed rats

Qian Zhang[a], Chuncao Xu[a], Shiqi Lin[a], Huanbin Zhou[a], Gangtao Yao[a], Hu Liu[b], Lili Wang[b], Xin Pan[a], Guilan Quan[a,*], Chuanbin Wu[a]

[a]*School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China*
[b]*School of Pharmacy, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada A1B 3V6*

Received 11 September 2017; received in revised form 27 October 2017; accepted 23 November 2017

KEY WORDS

Dissolving microneedles;
Acupoint;
Thymopentin (TP5);
Immune-modulating effects;
transdermal drug delivery;
Acupuncture

Abstract Dissolving microneedles carried drug molecules can effectively penetrate the stratum corneum of skin to improve the transdermal drug delivery. The traditional Chinese medicine acupuncture is based on the needle stimulation at a specific location (acupoint) to generate and transmit biochemical and physiological signals which alter the pathophysiological state of patients. However, the pain associated with conventional acupuncture needles and the requirement of highly trained professionals limit the development of acupuncture in non-Asian countries. The purpose of this study is to investigate whether the dissolving microneedles can be utilized as a self-administered painless replacement for acupuncture and locally released drug molecules can achieve expected therapeutic outcomes. Immunosuppressive rats were treated with acupuncture at Zusanli (ST36) acupoint using microneedles containing thymopentin. The immune functions and psychological mood of the immunosuppressed animals were examined. The proliferation of splenocytes was examined by CCK-8 assay. CD4 and CD8 expression patterns in spleen cells were detected by flow cytometry. The current study showed that use of either microneedles containing thymopentin or conventional acupuncture both resulted in immune cell proliferation, which was confirmed by flow cytometry. Furthermore, either conventional acupuncture or microneedles were able to effectively mitigate the anxiety caused by immune-suppression when applied on the ST36.

*Corresponding author. Tel.:fax: +86 20 39943115.
E-mail address: quanglan@mail.sysu.edu.cn (Guilan Quan).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2017.12.006

1. Introduction

The immune system plays an important role in protecting against diseases[1]. Immunosuppression can be caused by malignant tumors, infertility, cerebrovascular diseases, AIDS and other conditions[2] in which patient's immune function is weakened as evidenced by the decreased number of immunocompetent cells, abnormal cytokine expression and secretion and increased activities of immunosuppressive factors. As the onset of compromised immune function weakens the body's defenses, some fungi which are usually non-pathogenic for immunocompetent humans can cause severe infections in immunocompromised patients[3]. In addition, immunocompromised patients are often psychologically depressed[4,5].

Acupuncture is one of the important treatments of traditional Chinese medicine in China and some Asian countries and is becoming an alternative treatment in western countries for many diseases which are difficult to cure, including pain, asthma and major depression[6]. A large number of experimental and clinical studies confirmed that acupuncture could bidirectionally (up- and/or down-) regulate the immune system[1]. In the acupuncture treatment, the meridian and acupoints are the basis for effective acupuncture. Acupuncture treatment is actually the stimulation of the meridian and the acupoints. The acupoint injection[7], acupressure[8], electrical stimulation[9] and other a variety of alternatives of acupuncture are known to achieve a good clinical treatment effect.

Zusanli (ST36) is an important acupoint on the meridian of the human body and many treatments involve this point. It is an acupoint located at the posterolateral knee and about 5 cm (in human) under the fibula capitulum, and is related to the stomach, hence being named ST by the World Federation of Acupuncture-Moxibustion Societies[10]. According to the acupuncture meridian chart, immune functions could be regulated by the stomach and spleen which are the conceptual parts of human body and may not be exactly equivalent to anatomical organs defined by modern medicine. To treat and prevent diseases, acupuncture and moxibustion at ST36 are common practices in China and Japan, Koreas and Southeast Asian countries. A large number of studies have confirmed that acupuncture at ST36 can regulate immune functions which are both holistic and bidirectional, *i.e.*, achieving overall improvement of pathophysiological states by either up- or down-regulating patient's immune functions. It has been shown that acupuncture at ST36 can increase serum levels of immunoglobulin, lectins, hemolysin and antibodies, the number of antibody-forming cells and complements. ST36 has been widely used in the treatment of many conditions such as immunocompromised or immunodeficiency patients, and patients with hyperthyroidism disease. It has been shown that either needle stimulation of ST36 or injecting antiviral agents at ST36 can reduce the level of aminotransferase activity associated with hepatitis viruses in hepatitis patients. In addition, hepatitis viral infection leads to elevated levels of serum IgG by increasing total contents of complement C3, C4, B factor and decreasing immune complexes. It was shown that the overly expressed IgG was significantly reduced to a normal level and hepatitis B induced liver damage was repaired following acupuncture at ST36[11]. For older patients acupuncture at the ST36 point could increase their serum levels of IgA, IgG and IgM, thereby enhancing the immune functions to prevent the invasion of pathogens and to speed up recovery[1,12]. The stimulation at ST36 site was found to significantly increase the levels of white blood cells, neutrophils, erythrocytes and thrombocytes caused by conventional chemotherapy in patients with malignant tumors[13]. Therefore, acupuncture at ST36 can be an auxiliary treatment to alleviate side effects caused by chemotherapy and improve the quality of life of cancer patients. However, traditional acupuncture requires hypodermic needles that can cause needle phobia and generate biohazardous wastes. It also requires specifically trained personnel.

A novel microneedle-mediated transdermal drug delivery has received increasing attention in recent years[14]. Microneedle (MN) technology offers a distinctive method of cutaneous drug delivery. It painlessly pierces into the skin to administer drug in a minimally invasive and targeted manner[15]. Another advantage of MN is its potential for self-administration which can considerably increase patients' compliance[16]. Furthermore, MN avoids the first-pass effect, especially valuable for macromolecular biological agents such as proteins and polypeptides. Using MN technology, drug can be delivered through the stratum corneum of the skin in diversified forms such as drug-coated MNs, drug-encapsulated MNs, hollow MNs, or application of the drug on skin prior to being punctured with MNs[14,17–19]. Dissolving microneedle array (DMNA) patches, a new form of MN for transdermal delivery, dissolve in the body fluid as soon as the array is inserted into the skin, and the encapsulated drug is released as the needles dissolve. Moreover, DMNA made from biopolymers are low in cost and have no safety concerns associated with the risk of breakage like silicon and metal needle[20].

Therefore, the purpose of this study is to investigate whether the dissolving microneedles can be utilized as a self-administered painless replacement for acupuncture and locally released drug molecules with expected therapeutic outcomes. A thymic pentapeptide, thymopentin (TP5), was used as a model drug. TP5 loaded DMNAs composed of dissolving polymers were designed to percutaneously deliver TP5, an immune-stimulant, to the ST36 in immunosuppressed rats and the effects on immuno-response and behaviors of the animals were studied.

2. Methods

2.1. *Fabrication of DMNAs to encapsulate TP5*

DMNAs were prepared by a two-step micro-molding process described previously[21]. Briefly, the female mold was first made from polydimethylsiloxane (PDMS, Meryer Co., Ltd., Shanghai, China) by exactly inversely replicating the master structure of a copper-alloy made master mold as shown in Fig. 1. The female mold consisted of 100 (10 × 10) conical microcavities and the dimension of each cavity was 300 μm in base diameter and 800 μm in needle height. One gram of dextran (molecular weight 40,000, Aladdin Co. Ltd., Shanghai, China) was dissolved in 2 mL of deionized water to form a blank needle solution. To prepare TP5 (KaiJie Peptide Co., Ltd., Chengdu, China) loaded DMNAs, a solution containing 1 g dextran in 4 mL deionized water was prepared. TP5 (0.8 g) was dissolved in the solution to form TP5 loaded needle solutions. The base solution was prepared by adding PVP K90 (1.0 g) into 2.7 mL of ethanol and the preparation was left overnight. The blank needle solution or TP5 loaded needle solution prepared above was poured over the female mold, and then centrifuged at 4000 × *g* (Thermo Electron LED GmbH, Osterode, Germany) for 15 min at 4 °C to completely fill microcavities of the mold. Excess solution on the surface of the mold was removed. Next, the base solution was placed on the mold in which microcavities were already filled with the needle solution, then centrifuged at 4000 × *g* for 15 min and dried for 12 h at room

Please cite this article as: Zhang Qian, et al. Synergistic immunoreaction of acupuncture-like dissolving microneedles containing thymopentin at acupoints in immune-suppressed rats. *Acta Pharmaceutica Sinica B* (2018), https://doi.org/10.1016/j.apsb.2017.12.006