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Contents

In this first installment of a three part series, "The Art and Science of Traditional Medicine," we present a series of articles making a case for the integration of traditional Chinese medicine (TCM) into modern medical practice. From the new WHO Traditional Medicine Strategy to the application of systems biology in studying TCM, we aim to highlight the potential for creating an integrated, network-based health care system. The next two issues will cover herbal genomics and highlight the importance of quality control, standardization, regulation, and safety for traditional therapies. An overview of indigenous medicines in Europe, Africa, the Middle East, India, and the Americas will also be provided.

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Forewords

Articles

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The content contained in this special, sponsored section was commissioned, edited, and published by the *Science*/AAAS Custom Publishing Office. It was not peer-reviewed or assessed by the Editorial staff of the journal *Science*; however, all manuscripts have been critically evaluated by an international editorial team consisting of experts in traditional medicine research selected by the project editor. The intent of this section is to provide a means for authors from institutions around the world to showcase their state-of-the-art traditional medicine research through review/perspective-type articles that highlight recent progress in this burgeoning area. The editorial team and authors take full responsibility for the accuracy of the scientific content and the facts stated. Articles can be cited using the following format: [Author Name(s)], *Science* **346** (6216 Suppl), Sxx-Sxx (2014).

L. rhizome, an herb commonly used in Australia to treat symptoms of menopause and rheumatoid arthritis (*28*), and *Leonurus japonicus* Houtt., an herb commonly used in TCM for gynecology and obstetrics (*15*, *29*). Additionally, we previously found that ethanolic extracts from unprocessed main roots of *Aconitum carmichaeli* Debx. have potent in vitro profibrotic activities (*15*)–this is likely clinically relevant since consumption of *Aconitum* species is known to cause acute renal failure and has been recently linked to end-stage renal disease (ESRD) in a case report by the U.K. Medicines and Healthcare Products Regulatory Agency (*30*).

The impact of identifying and avoiding exposure to profibrotic botanicals is profound. For instance, about one-third of the Taiwanese population consumed AA-containing herbs between 1997 and 2003 (*31*), and AAN previously accounted for up to 10% of all ESRD in Taiwan (*32*). The banning of AA-containing herbs, together with other efforts such as public-awareness campaigns, education of patients, funding for research into chronic kidney disease, and provision of integrated care, has turned Taiwan into one of the few regions with retarded increase of ESRD incidence (*33*).

Moving forward

Due to the contradictory and complex roles botanicals play in fibrotic diseases, there is an urgent need for studies that investigate the efficacy, safety, and good practices for botanical-based remedies.

Since fibrotic diseases are multifactorial conditions and botanicals are typically multitarget entities, an efficacy-based strategy is particularly well-suited for studying antifibrotic botanicals (Figure 1).

Such a strategy is highly dependent on disease modeling. It is worth emphasizing that innovation is needed to develop high-quality in silico, in vitro, and in vivo models that can facilitate the investigation of antifibrotics and detect profibrotic activities.

Because evidence-based medicine is a relatively new concept in many countries (*34*), many clinical reports on herbal treatment of fibrotic diseases are criticized for poor quality. Diseases for which the literature has been recently reviewed include liver fibrosis (*35*, *36*), pulmonary fibrosis (*36*), multiple sclerosis (*36*), and adhesive small bowel obstruction (*37*). An efficacy-based strategy ultimately demands high-quality clinical trials to prove antifibrotic effects and invites interregional cooperation on pharmacovigilance of profibrotic botanicals, which is challenging due to the insidious nature of fibrosis and the variability in the distribution channels and legal status of botanicals across regions (*38, 39*).

Finally, traditional use is only an indication but certainly not a proof of either safety or efficacy (*40*). To harness and understand botanicals both as potential antifibrotic therapeutics and for the prevention of fibrotic diseases, future research and innovation must focus on efficacy and safety, and must be built on and contribute to good practices, which we have recently defined at length (*41*). Development and refinement of good practices, however, can only be achieved with sustainable funding.

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i-Needle: Detecting the biological mechanisms of acupuncture

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A long standing obstacle to the (full) integration and acceptance of acupuncture in conventional medicine lies in the difficulty of reconciling traditionally defined categories (acupoints, meridians, and energy flow or *qi*) with anatomical structures and biochemical pathways. Additionally, a unified scientific theory to explain the diverse effects of acupuncture (from pain control to immunomodulation) is lacking, despite important advances in the association of purinergic signaling with the effects of acupuncture on pain control. As new technologies simultaneously offer enhanced capacities to explore breadth (using 'omics) and depth (using nanobiochips) of biochemical events, we propose the innovative conjugation of these approaches into an intelligent needle (*i*-needle) as a means to overcome the abovementioned limitations.

Acupuncture is being widely debated in the medical community as a potential alternative or complementary treatment for many diseases (*1*). There are numerous challenges to achieving a consensus over the use of acupuncture in a medical environment, including: filling the gap in knowledge about the underlying molecular mechanisms of acupuncture, and (re)interpreting traditional categories (such as acupoints, meridians, and *qi*) and therapeutic indications within an evidence-based medicine framework. Important questions aimed at increasing our understanding of the molecular effects of needle stimulation have been posed, mostly regarding pain control (*2*), functional recovery of tissue (*3*), and immunomodulation (*4*), with remarkable work done as to the correlation of pain control with purinergic signaling (*5, 6*). Using 'omics-based technology and network representations, researchers have successfully mapped the molecular underpinnings of traditional categories (*7*). More generally, the holistic method used in acupuncture, which has long been difficult to reconcile with the scientific reductionist viewpoint, has recently been found to be compatible with a systems biology approach (*8*).

'Omics-based techniques are diverse and allow for the screening of targets from nucleic acids (DNA-sequencing, RNA-sequencing) to proteins and metabolites (mass spectrometry/liquid chromatography, nuclear magnetic resonance) and their heterogeneous interactions (chromatin immunoprecipitation-sequencing), to name just the major technologies. Recently, whole new areas of exploration have been opened with metagenomics and metatranscriptomics where the host-microbiome relationship can be analyzed systemically and in situ. Further, rapidly decreasing costs are permitting researchers to prefigure relatively high spatial (different body regions and tissues) and temporal resolution. Here, we propose to integrate such highly resolved molecular, temporal, and spatial data to reveal the molecular signaling pathways that flow from the tip of the needle to the disease/injury site.

Understanding the biochemical signaling pathway that the mechanical rotation of an acupuncture needle sets into motion (*9*) is an important starting point. Mechanosensing and mechanotransduction are widespread in biology with well-assessed relevance in embryonic development, i.e., type 1 epithelial-mesenchymal transition (EMT) (*10*). Their roles, however, have not been well explored under the broader definition of EMT (*11*)–which includes events such as wound healing (type 2 EMT) and cancer (type 3 EMT)–despite promising therapeutic results when mechanical stimulation is locally applied (*12*). Acupuncture needle stimulation (*9*) and low level laser therapy (*13*) are among the triggers that have been shown to initiate a series of synergistic events, including calcium waves, ATP fluxes (purinergic signaling), and changes in reactive oxygen and nitrogen species concentration, known to initiate healing (*14, 15*). The homeostatic effects of type 2 EMT include local changes in purinergic signaling, inflammation control, regeneration, and remodeling at the site of injury. By contrast, acupuncture is recommended for systemic diseases like rheumatoid arthritis (*1*) and is thought to act in a more global fashion.

Using the framework we propose here, we can investigate the long range, systemic effects of mechanotransduction by building on what has already been reported about the wound healing process, including the presence of peripheral markers of EMT (*16*).

To explore the long range effects of acupuncture, multiomic analysis of molecular events–occurring proximally (acupoint), distally from the stimulation point (target organ), and systemically (blood and gastrointestinal microbiome)–can be used to construct a spatial analysis (*17*). This information can then be enriched with data about the temporal onset of early gene expression, in addition to later time points (Figure 1A) to construct a systems biology view (network) of the biochemical events.

To build such networks and identify new targets for diagnosis and therapy, computational analysis must bring together the different 'omics approaches (Figure 1B), coupled with the requisite temporal and spatial resolution of the data (*19*). This type of network approach can identify the most important molecules from the thousands to tens-of-thousands of interactions and hundreds-to-thousands of molecules analyzed, also taking into account distal factors that might play a role in causing or modulating the pathologies.

Furthermore, the identification of additional markers is made possible with a complementary approach to the high

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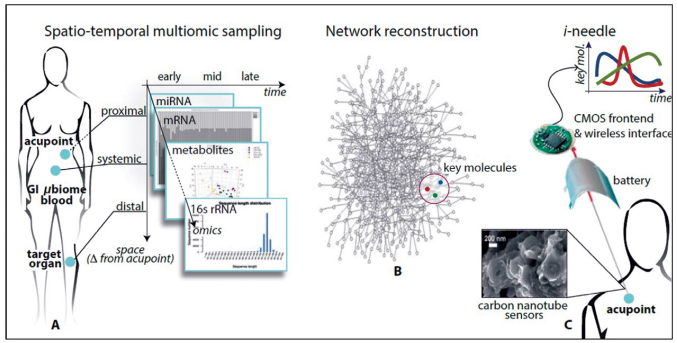

FIGURE 1. Elements of the i-needle. (**A**) A variety of 'omics techniques can be used to monitor molecular progress across body sites and over time. The pictured model shows the spatiotemporal multi-'omic sampling of the molecular flow of events over the therapy's delivery, from early molecular activation at the acupoint to the peripheral bloodstream and gastrointestinal (GI) microbiome (μbiome) to ultimately reach the target organ. (**B**) Multi-'omic systems biology enables the identification of a network of events, allowing interpretation of acupuncture in terms of a biochemical signaling flow that alters the whole system (body/patient). Network analysis and simulations allow identification of molecules that can be monitored as markers of the progress of the therapy (*18*). (**C**) Diagram showing carbon nanotube (CNT)-based sensor integration to continuously monitor the therapy-induced biochemical progression. Sensors are mounted on an energy-autonomous device that is able to transmit information remotely and in real-time.

throughput and low sensitivity of these 'omics analyses. This can be imagined in the form of a nanobiochip that is the size and shape of an acupuncture needle (hence, an "intelligent" needle or *i*-needle) (Figure 1C).

Toward this end, we recently created a proof-of-principle miniaturized platform, integrating revolutionary carbon nanotubes and nanographite petals, which can monitor five endogenous human metabolites using highly sensitive and selective nanobiosensors (*20*). The electronics needed to acquire and transfer the detected signal have already been sufficiently miniaturized (*21*) and can be powered by ultrathin polymer-based batteries (*22*) currently available on the market and able to meet the energy demands of the proposed *i*-needle (~80–130 μAh).

The challenge for the realization of the *i*-needle has already moved from the miniaturization to the integration step (*23*). Progress has already been made, based on recent reports of the measurement and transmission of temperature, pH, and endogenous metabolite data using single-platform enzyme-carbon nanotube hybrid sensors (*24, 25*) .

Conclusions

Overall, it is our hope that this research can provide a more unified approach to understanding the complex nature of patient responses to acupuncture—including effects as diverse as the control of pain, degeneration, and inflammation—and to addressing fundamental issues in acupuncture treatment, such as the frequency of delivery, developing more precise therapeutic indications, and establishing proper "dosage" guidelines. These steps will undoubtedly encourage acceptance of acupuncture as a complementary and/or alternative personalized treatment, with important application in a wide variety of areas including pain control, and degenerative and chronic inflammatory diseases, among others.

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Purinergic signaling in acupuncture

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The proposed role of purinergic signaling in the physiological basis of acupuncture was first presented in 2009. Data showing that ATP is released from keratinocytes and other skin cells during acupuncture treatments lends weigh to this hypothesis. ATP in turn activates P2X3 receptors on the sensory nerves in the skin, which then transmit those messages to motor neurons in the brain stem that control autonomic functions and modulate nociceptive activities. Here, we review and describe the recent evidence for purinergic signaling underlying acupuncture effects and propose ways to further test this hypothesis.

Introduction

It has been well established that adenosine 5′-triphosphate (ATP) is an intracellular energy source in cellular biochemistry. In 1970, Burnstock et al. suggested that ATP acted as a nonadrenergic, noncholinergic neurotransmitter in the gut (*1*), and in 1972 he named the extracellular actions of ATP, "purinergic signaling" (since ATP is a purine nucleotide), and formulated the purinergic signaling hypothesis (*2*).

In 2009, Burnstock proposed that purinergic signaling could be involved in the physiological mechanisms mediating acupuncture effects. This hypothesis suggested that mechanical deformation of the skin by needles or application of heat or electrical current leads to the release of large amounts of ATP from keratinocytes, fibroblasts, and other cell types in skin (Figure 1). The released ATP then activates P2X3 ion channel receptors on sensory nerves within the skin and tongue that transmit messages via sensory ganglia and the spinal cord to the brain stem and hypothalamus. These brain regions contain motor neurons that control autonomic functions, including cardiovascular, gastrointestinal, respiratory, and urinogenital activities—common targets of acupuncture treatments. These sensory neuron messages also modulate the pathways that lead to centers in the cortex responsible for conscious awareness of pain and other central nervous system activities, including sleep regulation (*3*). A number of subsequent studies have been published that also implicate purinergic signaling in various aspects of acupuncture, detailed below.

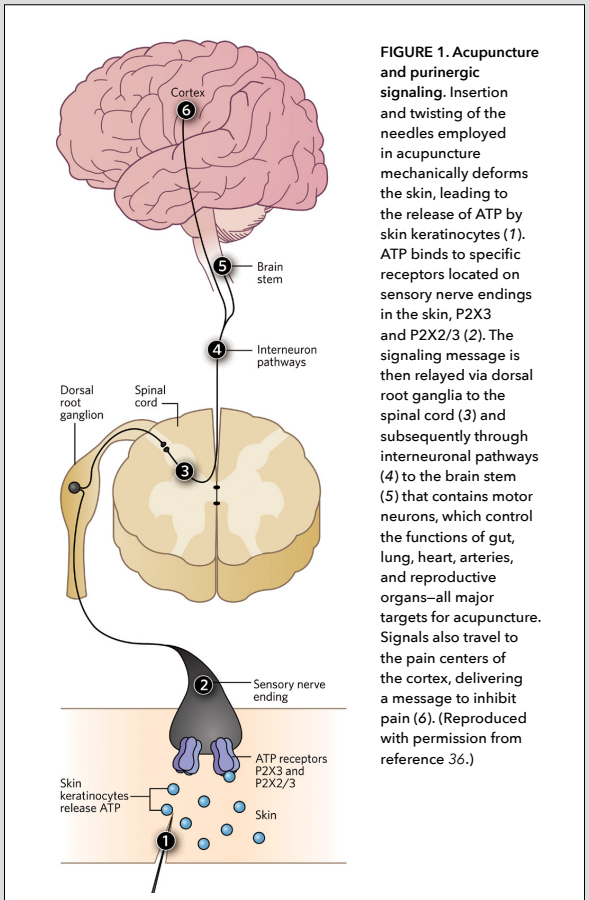

FIGURE 1. Acupuncture and purinergic signaling. Insertion and twisting of the needles employed in acupuncture mechanically deforms the skin, leading to the release of ATP by skin keratinocytes (*1*). ATP binds to specific receptors located on sensory nerve endings in the skin, P2X3 and P2X2/3 (*2*). The signaling message is then relayed via dorsal root ganglia to the spinal cord (*3*) and subsequently through interneuronal pathways (*4*) to the brain stem (*5*) that contains motor neurons, which control the functions of gut, lung, heart, arteries, and reproductive organs—all major targets for acupuncture. Signals also travel to the pain centers of the cortex, delivering a message to inhibit pain (*6*). (Reproduced with permission from reference *36*.)

Supporting evidence for the hypothesis

Studies that have established the components involved in the purinergic signaling pathway include: (i) release of ATP (in response to mechanical or chemical stimulation)

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