Developing influenza treatments using traditional Chinese medicine

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Humans have been faced the threat of epidemics such as influenza throughout their existence. Traditional Chinese medicine (TCM) practitioners began documenting their diagnostic and treatment principles related to epidemic diseases in the classic Chinese medical book, "Emperor Internal Medical Classic" (*1*). The unique treatments and herbal formulas used to combat influenza may serve as a source of information and inspiration for the development of new drugs (*2*).

Chinese herbal medicines and influenza

A major difference between Western and Chinese influenza treatments is the mode and targets of their actions. The first antiviral chemical drugs appeared in the West in the mid-1960s. Since then, many single-target therapeutics have been designed, but drug resistance is common. To circumvent this, Western medicine has incorporated multiple molecular targets into a single treatment using combination therapies, a practice now well accepted in the West.

Chinese herbal formulas (CHFs), on the other hand, often act via multiple modes that target not only the virus, but also various components of the host's immune response (Table 1), creating a synergistic effect. For example, *jinchai* capsules blunt viral replication by blocking adsorption of virions and preventing virus hyperalgesia-induced membrane fusion (*3*), while evodiamine blocks viral action by interfering with the AMPK/TSC2/mTOR signaling pathway, which is associated with virus-induced autophagy (*4*). Figure 1 summarizes the points of action of CHFs when treating influenza.

Isatis indigotica roots and influenza

Isatis indigotica roots (IIR) (*Banlangen*) have long been used to treat seasonal influenza in China. Currently, more than 100 chemical constituents of IIR have been identified. Among them, the compounds of epigoitrin; 2,4(1H,3H)-quinazolinedione; 4(3H)-quinazolinone; and clemastanin B have been demonstrated to kill or significantly inhibit the influenza virus. Studies from our laboratory have shown that polysaccharides extracted from IIR can prevent the influenza virus from attaching to host cell surfaces through a process involving hemagglutinins (*5*). Moreover, an indole alkaloid has been found to play a major role in preventing viral infection of host cells (*6*), while compounds derived from IIR can block translocation of the nucleocapsid protein at the early stage of replication, primarily through modulation of NF-κB signaling, thus inhibiting viral replication (*7*). In addition, IIR has been shown to exert immune modulatory effects in vitro and in vivo. In lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages, the methanolic extracts of IIR inhibited degradation of IκBα and production of nitric oxide, prostaglandin E_2, and interleukin (IL) 6 (*8*). The polysaccharides from IIR could promote proliferation of lymphocytes and macrophages, as well as production of IL-2 and interferon (IFN) γ in mouse models (*9*). Indirubin and its derivatives can suppress a number of pro-inflammatory cytokines/chemokines in infected human bronchial epithelial cells, human peripheral blood-derived macrophages, and alveolar epithelial cells (Table 1) (*10*, *11*). Taken together, these data imply that IIRs play a variety of roles protecting against viral infection by targeting both the virus and the host—a markedly different effect than that of marketed chemically synthesized drugs.

Drug development strategies using TCM

High-quality consistency, treatment effectiveness, safety assurance, and patient affordability are the key factors for drug development. TCM can inform research into these areas in the following ways.

Firstly, the strategies and principles underpinning the translational research used in TCM-based influenza treatments could be applied more broadly. Two possible approaches can be taken: the standard, bottom up bench-to-bedside strategy, or a more innovative approach that transitions empirical medical knowledge from TCM into an evidence-based research strategy. We proffer that the latter better reflects the real-world interaction between basic science and the TCM clinical experience.

Secondly, basic research and clinical studies on CHFs could be conducted in parallel. For example, the effects of extracts and/or combinations of the active compounds from commonly prescribed CHFs could be investigated concurrently with standardized clinical trials based on documented clinical experience.

Thirdly, well-defined methodologies for standardized assessment of the quality, efficacy, and safety of CHFs are still lacking. It is important to standardize the composition and level of active components of herbs in CHFs before including them in a basic research project or clinical trial so as to maintain the data integrity.

Finally, TCM research is complex. It therefore behooves all researchers to develop interdisciplinary, innovative, and collaborative research projects, through which the scientific foundation of TCMs can be elucidated and a new framework that incorporates modern medical science can be built.

We have been pioneers in an attempt to implement the abovementioned strategies using IIR, launching the first randomized control trial in China in 2010. Various 'omics

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TABLE 1. Examples of TCM and Western anti-influenza drugs.

Antivirals				Target	Target subject	Mechanism of action or therapeutic effect	Year Documented	Reference
Western anti-influenza compounds	Cyanovirin-N			Surface glycoproteins of enveloped viruses	Virus	Inhibits entry of virus	1997	*12*
	Nitazoxanide			Influenza hemagglutinin	Virus	Impairs hemagglutinin intracellular trafficking	2009	*13*
	DAS181			Influenza viral receptor	Host	Removes viral receptor	2006	*14*
	Amantadine/Rimantadine			Influenza M2 protein	Virus	Inhibits proton conductance of M2	1965	*15, 16*
	Favipiravir(T-705)			Influenza RNA polymerase	Virus	Inhibits viral RNA polymerase activity	2002	*17, 18*
	Oseltamivir/Peramivir/Zanamivir/ Laninamivir			Influenza neuraminidase	Virus	Blocks release of virus	1996/2000/ 1993/2005	*19-22*
Chinese anti-influenza herbal formulas, compounds, and constituents	Single herb	Ban Lan Gen (*Isatis indigotica* root)	Methanol extract	NF-κB signaling	Host	Inhibits nitric oxide and prostaglandin E_2 production, and NF-κB signaling in macrophages	1260s	*8*
			Polysaccharides	–	Host	Promotes transformation of lymphocytes and production of IL-2 and IFN-γ		*9*
			Clemastanin B	–	–	Blocks influenza ribonucleoprotein nuclear export, prolongs mean lifespan of infected mice		*7, 23*
			Indirubin	NF-κB signaling	Host	Interrupts virus-induced p38 MAP kinase activation and NF-κB translocation, and reduces expression of CCL5 in human bronchial epithelial cells		*10*
			Indirubin derivatives	–	Host	Suppresses pro-inflammatory cytokines and chemokines		*11*
		Da Qing Ye (*Folium isatidis*)	Monomer	–	–	Reduces mortality rate of influenza virus-infected mice	202–220 B.C.E.	*24*
		Jin Yin Hua (*Lonicera japonica*)	Ethanol extracts	Antiviral, immune-modulatory, and anti-inflammatory protein in mouse serum	Host	Reduces lung index and alleviates lung lesions in influenza virus-infected mice	1400s	*25*
		LianQiao (*Forsythia suspense*)	Ethanol and water extracts	–	Host	Regulates CCL5 and MCP-1 secretion in H1N1 virus-infected A549 cells		*26*
	Complex herbal formula	Ma Xing Shi Gan Tang + Yin Qiao San		–	–	Reduces time to fever resolution in patients with H1N1 influenza virus infection	2011	*27*
		Lian Hua Qing Wen capsule		–	–	Reduces time to fever resolution in patients with H1N1 influenza virus infection	2004	*28*

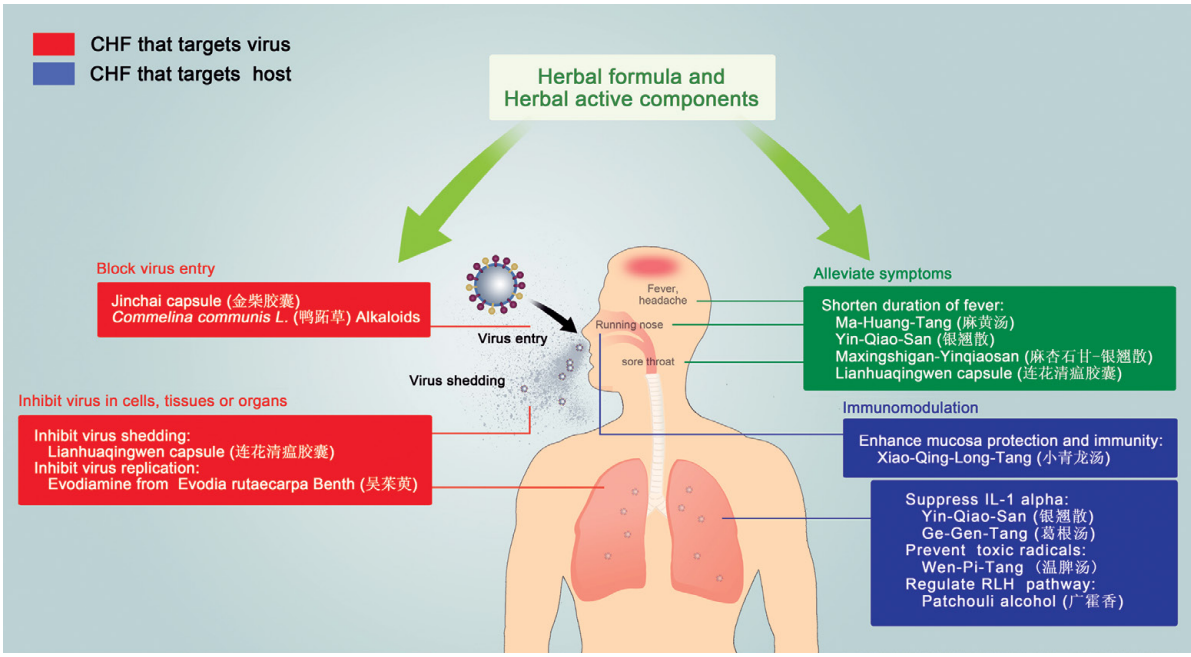

FIGURE 1. Holistic intervention to treat influenza.

technologies have been concurrently used to search for bioactive compounds, and we expect that additional active constituents with unique pharmaceutical activities will be found in the future. We have also combined the application of modern technologies with TCM clinical experience. For example, practitioners have noted that IIR appears to display beneficial clinical effects if administered during early onset of the disease (*9*). These studies suggest that further investigation of the mechanisms of IIR action is warranted. Importantly, using treatments with multiple sites of action may prevent or delay the generation of resistant viral strains.

References

1. J. Curran, G. P. Locum, Glasgow, *Brit. Med. J.* **336**, 777 (2008).
2. L. Jiang, L. Deng, T. Wu, *Cochrane Database Syst. Rev.* **3**, CD004559 (2013).
3. J. Zhong, X. Cui, Y. Shi, Y. Gao, H. Cao, *J. Tradit. Chin. Med.* **33**, 200 (2013).
4. J. P. Dai *et al.*, *PLOS ONE* **7**, e42706 (2012).
5. Z. Yang *et al.*, *Mol. Med. Rep.* **5**, 793 (2012).
6. Q. Zhu *et al.*, *Chinese Patent* CN 102836168 A (2012).
7. Z. Yang *et al.*, *Int. J. Mol. Med.* **31**, 867 (2013).
8. E. K. Shin, D. H. Kim, H. Lim, H. K. Shin, J. K. Kim, *Inflammation* **33**, 110 (2010).
9. Y. L. Zhao *et al.*, *Chin. J. Integr. Med.* **14**, 207 (2008).
10. N. K. Mak *et al.*, *Biochem. Pharmacol.* **67**, 167 (2004).
11. C. K. Mok *et al.*, *Antiviral Res.* **106**, 95 (2014).
12. B. R. O'Keefe *et al.*, *Antimicrob. Agents Chemother.* **47**, 2518 (2003).
13. J. F. Rossignol, S. La Frazia, L. Chiappa, A. Ciucci, M. G. Santoro, *J. Biol. Chem.* **284**, 29798 (2009).
14. M. P. Malakhov *et al.*, *Antimicrob. Agents Chemother.* **50**, 1470 (2006).
15. R. R. Grunert, J. W. McGahen, W. L. Davies, *Virology* **26**, 262 (1965).
16. J. R. Schnell, J. J. Chou, *Nature* **451**, 591 (2008).
17. Y. Furuta *et al.*, *Antimicrob. Agents Chemother.* **46**, 977 (2002).
18. Y. Furuta *et al.*, *Antimicrob. Agents Chemother.* **49**, 981 (2005).
19. C. U. Kim *et al.*, *J. Am. Chem. Soc.* **119**, 681 (1997).
20. Y. S. Babu *et al.*, *J. Med. Chem.* **43**, 3482 (2000).
21. M. von Itzstein *et al.*, *Nature* **363**, 418 (1993).
22. M. Yamashita *et al.*, *Antimicrob. Agents Chemother.* **53**, 186 (2009).
23. D. D. Sun, S. H. Yan, J. W. Chen, X. Li, L. W. He, *J. Nanjing Univ. TCM* **29**, 53 (2013).
24. Z. Liu, Z. Q. Yang, H. Xiao, *Virol. Sin.* **25**, 445 (2010).
25. H. Zhang, J. Song, J. Shi, *Chin. J. Chin. Materia Medica* **36**, 1071 (2011).
26. H. C. Ko, B. L. Wei, W. F. Chiou, *J. Ethnopharmacol.* **102**, 418 (2005).
27. C. Wang *et al.*, *Ann. Intern. Med.* **155**, 217 (2011).
28. H. X. Ouyang, Q. Y. Tang, Y. Z. Chen, Y. Wei, G. S. Li, *Chin. Med. Herald* **30**, 7 (2010).

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